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RELEASE OF MICROORGANISMS FROM SOLIDS AFTER SIMULATED HARD LANDINGS

FINAL REPORT

R. L. Olson, Ph. D.

S. J. Fraser

January 26, 1970

California Institute of Technology

Contract Number 952511

Prepared by

THE BOEING COMPANY
AEROSPACE SYSTEMS DIVISION
SEATTLE, WASHINGTON

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ABSTRACT

RELEASE OF MICROORGANISMS FROM SOLIDS AFTER SIMULATED HARD LANDINGS

Viable terrestrial microorganisms may be trapped in certain solid spacecraft materials and survive decontamination or terminal sterilization processes. These surviving organisms are then available for release into planetary environments if a hard impact occurs and the solid materials fracture. This investigation was conducted to determine the percentage release of microorganisms from the interior of solids after hard impact.

The effect of impact on microbial release and survival was investigated in each of the three test phases. During the first phase, the effect was studied by impacting internally contaminated methyl methacrylate pellets onto stainless steel. The second phase was carried out by impacting contaminated methyl methacrylate pellets into sand; while in the third phase, contaminated epoxy pellets were impacted onto stainless steel.

The methyl methacrylate data show the percentage of microbial release to be less than 1% at all four test velocities. An exception to this is seen in the epoxy results. The percentage of total microbial survival after impact is velocity dependent but independent of initial spore concentration. The fact that the total number of organisms surviving impact decreases as the velocity increases is of significance. This decrease in total survivors with an increase in velocity, offsets an otherwise expected increase in released viable organisms as material fracturing increases with velocity.

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RELEASE OF MICROORGANISMS FROM SOLIDS AFTER SIMULATED HARD LANDINGS

SUMMARY

RELEASE OF MICROORGANISMS FROM SOLIDS AFTER SIMULATED HARD LANDINGS

This is the final report submitted by The Boeing Company to the Jet Propulsion Laboratory on JPL Contract Number 952511. Three research phases were included in the contract that extended from May 1969 through February 1970. These phases are discussed separately in this report in Section II, III and IV respectively. A summary of the total contract effort is presented in Section I. Section I also includes applicable data which were obtained from previous Boeing supported investigations.

1.0 INTRODUCTION

Viable terrestrial microorganisms may be trapped in certain solid spacecraft materials and survive decontamination or terminal sterilization processes. These surviving organisms are then available for release into planetary environments if a hard impact occurs and the solid materials are fractured. The objective of this investigation was to determine the percentage release of microorganisms from the interior of solids after hard impact. Data of this nature are required in order to evaluate the probability of microbial release from spacecraft within the planetary contamination constraints established by the Committee on Space Research (COSPAR) and the National Aeronautics and Space Administration (NASA).

The probability of release can have a significant impact on the spacecraft terminal sterilization cycle as well as the flight acceptance cycles by its inclusion in a total probabilistic systems analysis of contaminating events. This study provides information that can be used in probability calculations related to planetary quarantine.

2.0 TECHNICAL DISCUSSION

The effect of impact on microbial release and survival was investigated in each of the three test phases. During the first phase, the effect was studied by impacting internally contaminated methyl methacrylate pellets onto

stainless steel. The second phase was carried out by impacting contaminated methyl methacrylate pellets into sand; while in the third phase, contaminated epoxy pellets were impacted onto stainless steel. As previously stated, detailed descriptions of these tests are presented in Sections II, III and IV.

Pellets were prepared by inoculating liquid methyl methacrylate and Eccobond epoxy with controlled numbers of Bacillus subtilis var. niger spores. The plastic materials were then polymerized into solids. Pellets, weighing approximately 1 gram, were fabricated from the seeded material and surface sterilized. Randomly selected pellets were analyzed to establish the mean spore number per gram of solid material before launching.

The tests were performed by firing the internally contaminated pellets at four velocities into sterile collection canisters. Pellet launching velocities were chosen to encompass the velocity with which a planetary vehicle would hard impact the Martian surface during an entry from orbit or a direct entry. The selected velocities were 550, 1500, 3100 and 5100 feet per second. Impact occurred on stainless steel or in sand mounted in a collection canister. After impact, the pellet fragments were assayed to determined: (1) the number of viable spores that were released on the surfaces of the particles from the pellet interior; or (2) the total number of organisms that survived impact on and in the particles.

The number of released spores was established by recovering the pellet particles from the collection canister, embedding them directly into molten Trypticase Soy Agar, and incubating them at 30°C for two weeks. Daily microscopic scanning of the incubating particles facilitated the detection of early colony developments on the fragmented surfaces. Each colony thus observed was recorded as one released spore.

The total number of spores that survived impact on and in the pellet particles was determined by utilizing two different recovery techniques. Methyl methacrylate pellet particles were collected and dissolved in acetone and the spores were recovered by membrane filtration. The Eccobond pellet particles were collected and subjected to a wet grinding procedure for pour plate analysis since a suitable solvent was not available for the epoxy. The counts obtained

from these two procedures were recorded as the total number of organisms surviving impact.

Similar recovery procedures, to those just described for obtaining survival after impact data, were used to determine the number of organisms present in pellets before firing. Methyl methacrylate was dissolved and the epoxy was wet ground. In this case the intact pellet was processed rather than particles. The counts obtained by this procedure were recorded as the initial number of organisms present before firing.

3.0 RESULTS AND CONCLUSIONS

Test firings at the beginning of each of the three study phases determined the weight percentage of the pellet recovered. A hard impact on stainless steel surfaces always produced pellet shattering at all velocities. Conversely, impact into sand only resulted in pellet fracturing at 3100 and 5100 ft/sec. Pellets impacted in sand at 550 and 1500 ft/sec exhibited limited areas of abrasion.

The procedure employed to recover the fractured particles from the impact canister accounted for virtually all of the pellet weight after impact. An exception to this was those pellets impacted at 5100 ft/sec on stainless steel (Table 1). The 5100 ft/sec velocity appeared to vaporize some of the pellet material producing a "cloud" in the gun chamber. Agar plates to monitor for fallout and a filter air sampling device were placed in the gun chamber to determine if viable organisms were present in the "cloud." No viable microorganisms were detected. This indicates that the vaporized pellet material obtained from 5100 ft/sec firings is not a significant factor with respect to release.

Survival data are based on the total number of viable spores recovered from the surface and interior of projectile fragments after impact. These data are presented in Table 2. A statistical analysis of the data for methyl methacrylate impaction on stainless steel indicates that there is not a significant difference at the 5% level of significance in the percentage of spore survival with respect to inoculum level. However, there is a highly significant difference when the effect of velocity is considered. The epoxy and methyl

Table 1: MEAN PERCENT RECOVERY OF PELLET MATERIAL AFTER IMPACT

Firing Velocity, Ft/Sec	Methyl Methacrylate Impacted Onto Stainless Steel	Methyl Methacrylate Impacted Into Sand	Eccobond Impacted Onto Stainless Steel
550	99 (a)	100	97
1500	94	100	95
3100	87	98	91
5100	66	100+	61

(a) Mean of 6 replicates; percentage by weight

Table 2: MEAN PERCENT SPORES SURVIVING IMPACT IN INTERNALLY CONTAMINATED MATERIALS

Ft/Sec	Methyl Methacrylate Impacted Onto Stainless Steel				Methyl Methacrylate Impacted Into Sand	Eccobond Impacted Onto Stainless Steel
	(Number of Spores Initially Available for Release Per Gram Pellet)					
	10^2 (a)	10^3 (b)	10^4 (b)	10^5 (a)	10^4 (a)	10^3 (a)
550	63 (c)	95	86	82	100+	81
1500	54	52	49	69	98.8	80
3100	5	10	13	9	66.1	91
5100	0.2			0.7	18.5	6

(a) JPL Contract research
(b) Boeing supported research
(c) Mean of 6 replicates

methacrylate sand impact results appear to follow the same general trend with respect to velocity.

These data show that as the velocity increases, the percentage of surviving organisms decreases. These survival data are important then in evaluating the results from the microbial release tests. They are also of interest when considering other aspects of the planetary quarantine problem. Since viable spores are shown to be present after impact at all velocities tested, they would be available for release into the planetary environment by such mechanisms as erosion.

The results of the percentage of microbial release after impact are given in Table 3. A statistical analysis of all the methyl methacrylate data indicate that there is no significant difference in the percentage of spores released at the four test velocities. There are, however, significant differences between the various inoculum levels. Less than 1% of the initially available spores was released on impact into stainless steel and less than 0.1% in sand.

The Eccobond epoxy data showed a significantly higher percentage of release. These latter results are not comparable to any of the data obtained from methyl methacrylate. Therefore, it appears that either the microbial release characteristics of Eccobond are significantly different from those of methyl methacrylate or problems exist in the assay procedure.

In order to accurately calculate the percentage of microbial release from Eccobond at hard impact, the total number of spores available for release must be established. Since epoxy resins are impervious to solvents, a potential problem exists when enumeration of pellet spore levels is performed by pellet grinding. The number of spores added to the Eccobond during fabrication totaled 10^5/gram epoxide. After pellet fabrication, the grinding procedure showed the spore level/gram pellet to be approximately 10^3. However, a 10^5 inoculum level, if used to seed methyl methacrylate, will show an approximately one log reduction when the spores are recovered by plastic dissolution.

The possibility that pellets were consistently launched with a higher spore level than the 10^3/gram established by grinding must be considered when evaluating

Table 3: MEAN PERCENT SPORES RELEASED FROM INTERNALLY CONTAMINATED MATERIALS

Ft/Sec	Methyl Methacrylate Impacted Onto Stainless Steel				Methyl Methacrylate Impacted Into Sand	Eccobond Impacted Onto Stainless Steel
	(Number of Spores Initially Available for Release Per Gram Pellet)					
	10^2 (a)	10^3 (b)	10^4 (b)	10^5 (a)	10^4 (a)	10^3 (a)
550	0.6 (c)	0.1	0.2	0.03	0.001	6.2
1500	0.4	0.6	0.6	0.3	0.001	22.9
3100	0.6	0.8	0.1	0.02	0.06	12.7
5100	0.2			0.002	0.06	1.1

(a) JPL Contract research
(b) Boeing supported research
(c) Mean of 6 replicates

data such as shown in Table 3. The relatively high percentage of microbial release may be explained if, in actuality, more spores were available for release than the assay procedure showed. Even a one log increase in numbers initially present would decrease the percent released by a factor of ten. The results then would be more consistent with methyl methacrylate data on the percentage of microbial release from solids.

The probability of microbial release can be calculated from the data presented in this report. This probability is dependent on the total number of organisms available for release. Therefore, in order to be meaningful, release probabilities must be calculated for specific cases once the total microbial load has been established on a spacecraft.

Another consideration is log reduction. The methyl methacrylate data show a minimum of a two log reduction from initial numbers to released organisms at all test velocities. This is equivalent to two D-values (D = 5 hrs for encapsulated organisms) and therefore could influence the sterilization cycles. The Eccobond epoxy release results show a one log reduction at the four velocities tested. However, until the apparent problems associated with the epoxy assay technique have been resolved, these results must be interpreted with great care.

In summary, the methyl methacrylate data show the percentage of microbial release to be less than 1% at all test velocities. An exception to this is seen in the epoxy results. The percentage of total microbial survival after impact appears to be velocity dependent but independent of initial spore concentration. The fact that the total number of organisms surviving impact decreases as the velocity increases is of significance in interpreting the release data. This decrease in total survivors with an increase in velocity, offsets an otherwise expected increase in released viable organisms as fracturing of materials increases with velocity. The data obtained during this study indicate that the probability of microbial release should probably be re-evaluated with respect to the currently established COSPAR requirements.

4.0 RECOMMENDATIONS

The results obtained from this program provide valuable data on microbial release and indicate areas where additional research would be advantageous.

It is recommended that three additional investigations be initiated to enable a more refined and expanded interpretation of microbial release data for planetary contamination assessment. The recommended studies are outlined below.

1. Grinding Efficiency Studies for Microbial Recovery

   This investigation would provide the required information to fully interpret the Eccobond epoxy data by determining the actual microbial level in pellets before firing. The objective would be to determine the efficiency of the grinding technique used to recover bacterial spores from internally inoculated Eccobond epoxy for enumeration. In addition, the information developed could be used in assaying other solid materials for enumeration.

2. Impaction of Inoculated Eccobond Into Sand

   During the reported study, data were obtained on microbial release from methyl methacrylate impacted into sand. It is desirable to obtain similar data on a second material. This study would provide supplemental information that would afford a more meaningful interpretation of results on the release of microorganisms from diverse solid materials impacted into sand.

3. Erosion of Inoculated Methyl Methacrylate and Eccobond by Sand Blasting

   Since during this study it was shown that microorganisms survived hard impact in the interiors of fragmented particles, it is desirable to know if the organisms would be released by the effects of erosion on a planetary surface. This investigation would determine the effect of sand erosion on the release of bacterial spores from methyl methacrylate and Eccobond.

When these three investigations have been completed, data will be available to: (1) fully interpret the epoxy percentage release results obtained for the contract; (2) correlate and substantiate the release percentages obtained with methyl methacrylate and Eccobond epoxy impacted in sand; and (3) evaluate the effect of sand erosion on the release of microorganisms that do survive impact in particles.

SECTION II

SECTION II

TEST I

RELEASE OF MICROORGANISMS FROM METHYL METHACRYLATE IMPACTED ON STAINLESS STEEL

TEST I

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TEST I

RELEASE OF MICROORGANISMS FROM METHYL METHACRYLATE IMPACTED ON STAINLESS STEEL

1.0 PURPOSE

The investigation was conducted to determine the percentage release of microorganisms embedded in pellets that were impacted on stainless steel plates.

2.0 INTRODUCTION

Methyl methacrylate pellets were fabricated so that each pellet interior contained approximately 10^2 or 10^5 Bacillus subtilis var. niger spores. A series of pellets were propelled from a gun at 550, 1500, 3100 or 5100 feet per second, respectively. Impact occurred on a stainless steel plate mounted in a collection canister. After impact, the pellet fragments were analyzed to determine the number of exposed viable microorganisms and the effect of impact upon the survival of the spores in the interior of the particles. The test program is presented in Table

3.0 PROCEDURE

3.1 PELLET MANUFACTURE

3.1.1 Preparation of Spore Stock

A 0.1 ml aqueous suspension of 10^9/ml of Bacillus subtilis var. niger spores was placed in the bottom of sterile planchets. The stock spore suspension was a minimum of 6 months old. The planchets were dried overnight by airflow from a Class 100 clean bench. When all the water had evaporated, 10 planchets were placed in a bottle containing 30 ml of ethanol and insonated for 20 minutes. The level of the bath water was adjusted so that it was halfway up the side of the bottle. After insonation, the 30 ml spore-ethyl alcohol suspension was pipetted into a sterile, capped bottle. All planchets were rinsed in fresh ethanol and the washings plus additional ethanol was combined with the 30 ml stock to bring the total to 100 ml.

Table 1: TEST PROGRAM - METHYL METHACRYLATE PELLETS IMPACTED ON STAINLESS STEEL PLATES

Velocity Feet/Second	Analysis for Total Percent Pellet Recovery (unseeded)	Analysis for Spores Released After Impact		Analysis For Total Surviving Spores After Impact		Procedural Controls		Analysis for Initial Spore Level Before Firing	
		IA [a]	IB [b]	IA	IB	IA	IB	IA	IB
550	6 [c]	6	6	6	6	3	3	3	3
1500	6	6	6	6	6	3	3	3	3
3100	6	6	6	6	6	3	3	3	3
5100	6	6	6	6	6	3	3	3	3
	24	24	24	24	24	12	12	12	12

Total: 168 Pellets

a 10^2 spores per gram pellet

b 10^5 spores per gram pellet

c Number of replicate pellets

The stock inoculum was refrigerated until used. A plate count of the spore stock was performed 1, 3 and 7 days after preparation to accurately determine the number of spores per ml.

3.1.2 Inoculation of Methyl Methacrylate Powder

Forty grams of methyl methacrylate powder were placed in a sterile 150 mm petri plate. The desired number of spores plus enough ethyl alcohol to bring the volume to a total 40 ml per 40 grams of methyl methacrylate powder was added to the powder.* The mixture was then manually stirred with a wooden applicator stick. The wet seeded powder was air dried in a Class 100 clean bench overnight. The dried seeded powder was sifted through a 40 mesh screen and stored in a glass beaker. All seeded powder was used within one week of preparation.

3.1.3 Removal of Preservative From Liquid Methyl Methacrylate

One hundred ml of liquid methyl methacrylate was placed in a clean 250 ml separatory funnel. A 100 ml freshly prepared 2% solution of sodium hydroxide was then added. The mixture was gently swirled for one minute and allowed to separate into two fractions. The bottom pink fraction containing the preservative was discarded. Another 100 ml of 2% sodium hydroxide was added, and the washing process repeated until no pink color was observed in the bottom layer. When the bottom layer was clear, one additional washing with 100 ml sodium hydroxide was performed. The washed liquid methyl methacrylate was rinsed with separate 100 ml volumes of distilled water to remove all traces of sodium hydroxide. The number of rinses was one more than the sodium hydroxide. All water (bottom layer) was drained from the funnel. The washed liquid methyl methacrylate was used within 24 hours of preparation.

3.1.4 Plastic Fabrication

Forty ml of washed liquid methyl methacrylate were added to 40 gm of seeded

* Test IA pellets were inoculated to effect a final level of 10^2 spores per gram of pellet.

Test IB pellets were inoculated to contain approximately 10^5 spores per gram of pellet.

powder in a glass beaker and mixed with a wooden applicator stick until a uniform slurry was obtained. The mixture was poured into glass test tubes (13 x 100 mm) until they were 2/3 full. As each tube was filled, the beaker was swirled to assure a homogeneous mixture. When all tubes were filled, they immediately were placed in a desiccator jar and the pressure reduced to 5 inches of mercury for 10 minutes. The vacuum was sufficient to create a slow bubbling action of the mixture in the tubes, but not so low as to cause the mixture to "climb" out the tube as the air was removed by the vacuum. The tubes were transferred for curing to a 50°C water bath and heated for 1.5 hours. The water level of the bath was adjusted so that it was slightly above the plastic level in the tubes. After 1.5 hours the tubes were removed from the bath and allowed to cool at room temperature for 10 minutes. The fabricated plastic rods were removed by breaking the glass tube. The rods were then stored in a glass jar in the freezer (-18°C) until they were machined into pellets.

3.1.5 Pellet Machining

A seeded methyl methacrylate rod was machined into firing pellets by turning the rod on a small bench lathe. Each finished pellet was approximately 0.32 inches in diameter, 0.64 inches long, and weighed approximately 1.0 gram. The machining sequence was to cut all rods of the same plastic batch to the established diameter. Face cuts were then made to finish the pellets to the correct length. The time necessary to machine the methyl methacrylate rods into finished projectiles did not exceed 8 hours for each plastic batch (40 grams powder plus 40 grams liquid).

3.1.6 Pellet Surface Sterilization

After machining, each pellet was identified by a number, weighed to the nearest one-hundredth of a gram and this information recorded. Each pellet was surface sterilized in a freshly prepared 2,000 ppm chlorine solution for 10 minutes. This was followed by a 10 minute soak in a fresh filter-sterilized 2% solution of sodium thiosulfate. Each pellet was stored in a freezer in a sterile, appropriately labelled, screw-capped test tube until launched.

The maximum time a projectile was at room temperature, from weighing to final storage in the freezer, did not exceed 2 hours.

The standardization of the time that seeded plastic was exposed to room temperature was established to avoid any large differences in spore levels for each pellet batch. Previous data indicated a one log$_{10}$ reduction occurred in spore populations if seeded plastic was kept at room temperature for periods exceeding 3 days. Therefore, the total elapsed time from plastic fabrication, through pellet machining, weighing, surface sterilization to final freezer storage did not exceed 24 hours at room temperature for each projectile.

3.2 PELLET LAUNCHINGS

3.2.1 Collection Canister

The sterile collection canister (Figure 1) was constructed so that an impacting pellet struck the slanted stainless steel witness plate and all pellet particles remained trapped in the canister.

3.2.2 Pellet Launching

The pellets were fired by personnel of the Damage Mechanics Laboratory. In order to assure reliability in the firings, one person was responsible for all launchings. The gun (Figure 2) was cleaned before each day's firing by launching 2 sterile pellets to flush the barrel and chamber. The sterile collection canister containing the target of stainless steel was positioned in the gun chamber. The pellets were loaded in the breech using sterile forceps. The cotton plug in the canister was removed, the chamber door sealed, the pressure established at the desired level, and the pellet launched. After firing, the gun chamber pressure was equalized to atmospheric pressure with cotton filtered air. The chamber door was opened, the cotton plug replaced in the canister, the velocity recorded, and the canister returned to the Microbiology Laboratory for analysis.

The methyl methacrylate pellets were fired at velocities of 550 ($\pm$ 100),

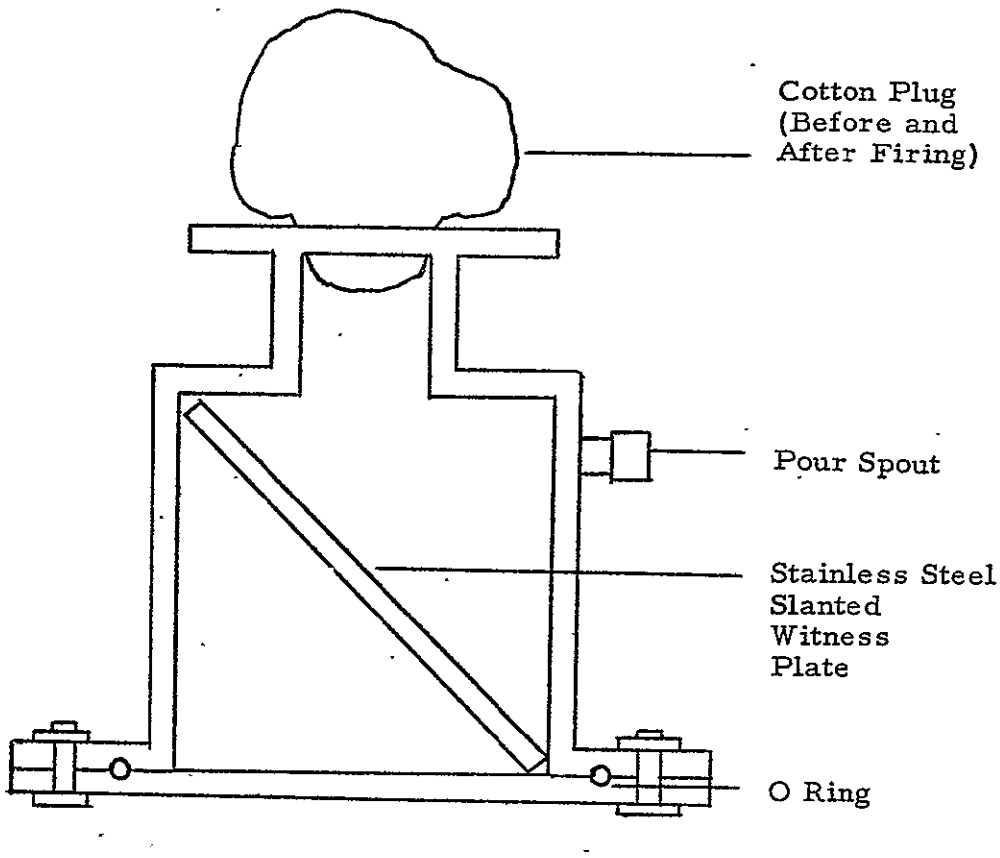

Figure 1: COLLECTION CANISTER

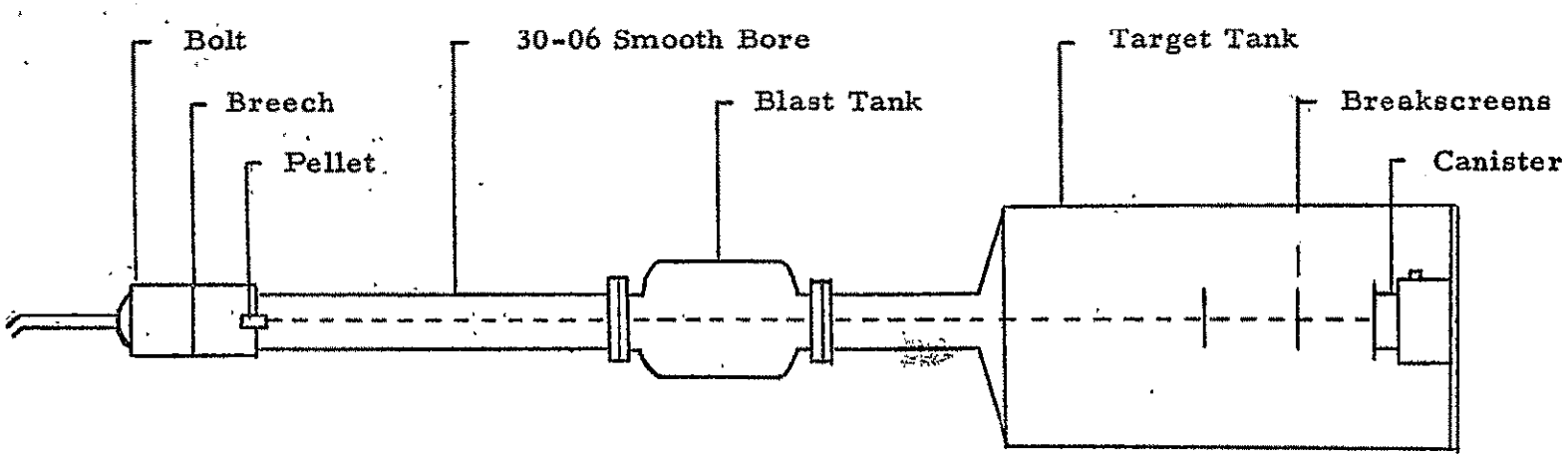

Figure 2: GAS AND POWDER GUN

1500 (±200), 3100 (± 300), and 5100 (± 300) ft/sec. Compressed air was employed to attain 550 ft/sec and the pellet fired into a chamber maintained at atmospheric pressure.

A powder charge was used to launch pellets at 1500, 3100, and 5100 ft/sec. Ambient chamber conditions were maintained for the 1500 ft/sec velocity while 3100 and 5100 ft/sec launchings occurred at 500 mm pressure. Previous test data established that these pressures were necessary to ensure complete burning of the powder charge used for launching. Target velocities were measured using breakscreen techniques.

A minimum of seven pellets were fired each day of Day 1, 2, 3, and 4 of a five day working week. Each day 6 seeded pellets were impacted in separate collection canisters. An analysis for spores released was conducted on 3 of the pellets and an analysis for total spore (number surviving impact was performed on the remaining 3 pellets. An unseeded control pellet was fired and processed using the same analytical techniques. This last pellet launched each firing day established the reliability of the test data with respect to possible contamination due to procedural techniques.

3.3 PELLET ANALYSIS

3.3.1 Percent Pellet Recovery After Impact

Six unseeded methyl methacrylate pellets were launched at each test velocity and impacted on stainless steel. Each pellet was weighed, fired, and collected in a separate canister. All particles from the impacted pellet were weighed again. The procedures used to recover the plastic particles were identical to those employed in the analysis for released spores from seeded pellets after impact. The results of these firings established the percent of pellet recovered. The particles of each impacted pellet were saved and delivered to JPL.

3.3.2 Analysis for Spores Released After Pellet Impact

The collection canister containing the impacted pellet was analyzed in the Microbiology Laboratory. Analysis for the recovery of released spores was performed as outlined in Figure 3.

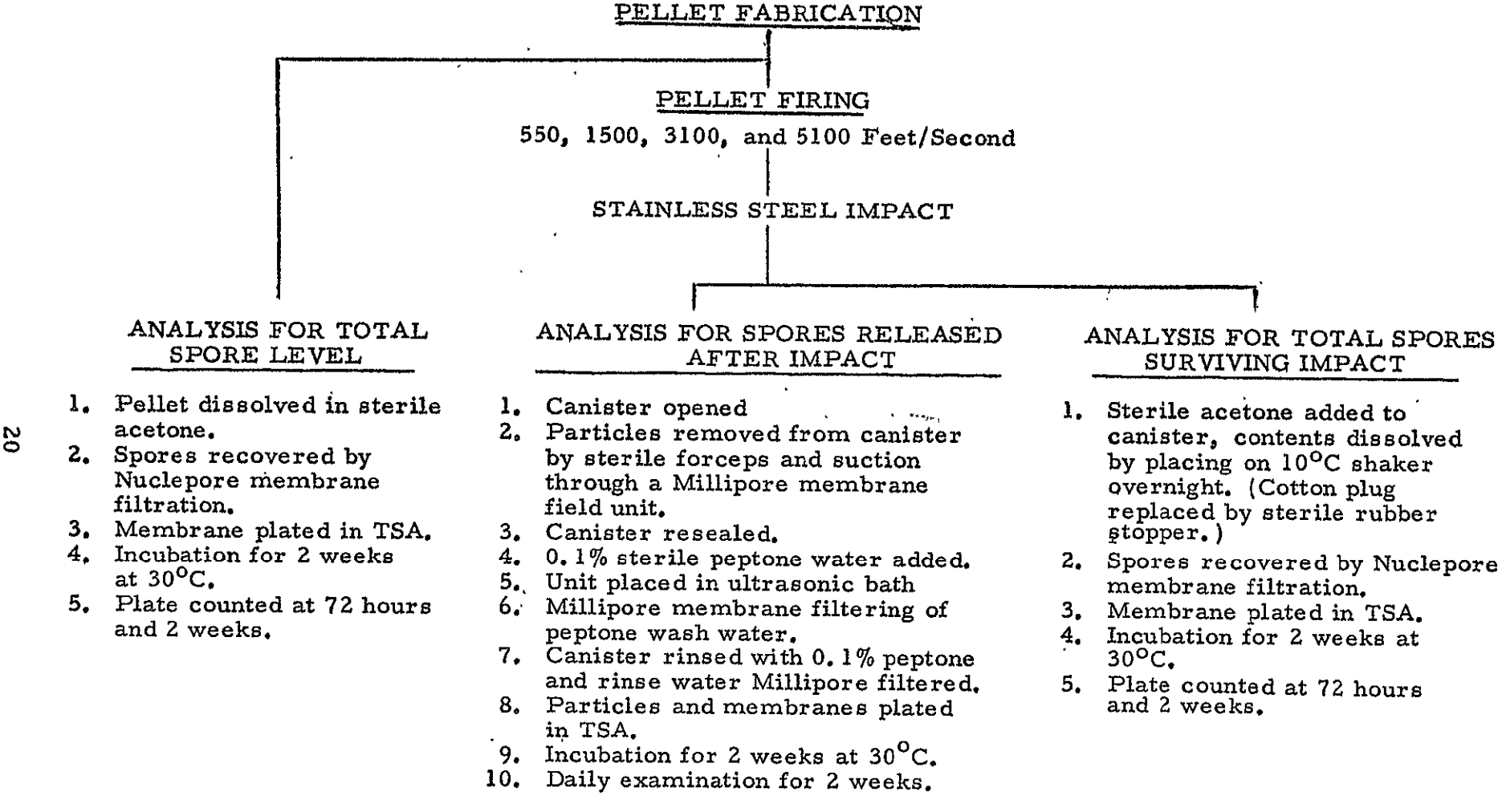

Figure 3: PELLET ANALYSIS

The collection canister was opened in a Class 100 clean bench. Large pellet particles were transferred, with sterile forceps, to a sterile petri plate. Trypticase Soy Agar (TSA) was then poured into the plate so that each particle was completely covered with the medium. Small particles were recovered from the canister by utilizing suction through a Millipore membrane field monitor unit. After thoroughly vacuuming the inside of each canister part, the Millipore unit was separated and the particles on the filter plated. In addition, TSA was poured into the Millipore unit and allowed to solidify.

The collection canister was reassembled and filled with 800 ml of sterile 0.1% peptone water. The cotton plug was replaced in the canister and the unit placed in an ultrasonic bath so that the level of bath water was slightly above that of the peptone water. The canister was insonated for 30 minutes. The canister was removed from the bath, the pour spout opened, and the peptone wash water filtered through a Millipore membrane. The canister was rinsed with additional 0.1% peptone and the rinses also filtered. The canister was then opened and examined carefully for any remaining pieces of pellet. All membranes, particles and field monitoring units were incubated in TSA for 2 weeks at 30°C. Daily examination was performed and each colony observed was recorded as one released spore.

3.3.3 Analysis for Total Number of Spores Surviving Impact

Analysis for the recovery of the total number of spores surviving impact was performed as outlined in Figure 3. The cotton plug was removed and 800 mls of sterile acetone added to the canister. The unit was re-sealed with a sterile rubber stopper and placed on the shaking platform in a 10°C refrigerated incubator. The unit was shaken overnight to dissolve all particles in the acetone. When complete dissolution occurred, the acetone was filtered through a Nuclepore filter to recover the spores. The canister was rinsed with sterile acetone and the rinse acetone filtered. The membranes were impinged upside down in molten TSA and incubated for two weeks at 30°C. At 72 hours an initial colony count was made and additional observations made throughout the remainder of the incubation period. The spores counted were recorded as the total number of spores surviving impact.

3.3.4 Analysis for Total Spore Level

On each day of firing, 2 pellets were chosen at random, dissolved in acetone, and the total spore level determined. The pellet was placed in a one liter screw-capped bottle containing sterile acetone. The contents of the bottle were agitated overnight on a 10°C shaker. Filtration through a Nuclepore membrane recovered the spores from the acetone. The membrane was impinged upside down in molten TSA and incubated at 30°C for 2 weeks. At 72 hours an initial colony count was made and additional observations made throughout the remainder of the incubation period. This procedure established the number of spores present per gram of pellet.

3.3.5 Procedural Controls

On each test day, an unseeded control pellet was launched after the seeded pellets were fired. The control pellet particles were subjected to appropriate analysis as outlined in Figure 3. This control pellet established the reliability of the test data with respect to possible contamination due to procedural techniques.

Also, on each test day, a seeded pellet was selected at random and embedded in melted TSA. The plate was incubated at 30°C and examined periodically for 2 weeks. The absence of surface colonies established reliability in the method used to sterilize the pellet surface.

3.4 DATA RECORDING AND ANALYSIS

The data was recorded on data sheets that contained the date of pellet firing, pellet number and weight, the velocity of impact, the analysis performed and the results of the analysis. The data sheet for each test day was checked and initialed by the program manager.

These data provided information on:

(1) the number of viable organisms released from solids after hard impact;

(2) the percentage of viable microorganisms surviving impacts at the test velocities;

(3) differences in release and/or survival of organisms due to variations in impact velocities.

The data was evaluated statistically using analysis of variance techniques. These analyses detected any statistically significant difference between pellets in a replicate group and between pellets impacted at 550, 1500, 3100 and 5100 ft/sec.

4.0 RESULTS AND DISCUSSION

4.1 PERCENT PELLET RECOVERY AFTER IMPACT

The percentage of plastic material recovered from the canister after pellet impaction at the four test velocities is presented in Table 2.

Table 2: PERCENT RECOVERY OF PELLET FOLLOWING IMPACT

Velocity Ft/Sec	Mean Percent Recovery of Pellet (a)
550	99
1500	94
3100	87
5100	66

(a) Mean of 6 Pellets

At the 550 and 1500 ft/sec, virtually all of the pellet was recovered. At the higher velocities, 3100 and 5100 ft/sec, only 87 and 66% of the initial weight of the pellets could be reclaimed. These particles showed a great deal of bubbling. It was concluded that the weight loss and bubbling was due to the heat generated when the pellet impacted on the stainless steel.

At 550 ft/sec, the pellets cracked into approximately six pieces. The 1500 ft/sec velocity resulted in a completely fractured pellet. The number of particles produced from one pellet averaged about 60 and the pieces showed sharp, clean fracture edges. An impact velocity of 3100 ft/sec yielded particles that were almost entirely composed of bubbles. The pellet pieces appeared frothy and opaque. Particles obtained from a 5100 ft/sec impacted

pellet were dark and resembled semi-charred bits of plastic.

Figures 4, 5, 6 and 7 show a comparison of the various types of pellet fracturing at the four test velocities.

4.2 SPORE RELEASED FROM THE INTERIOR OF IMPACTED PELLETS

The percentage of spores released from the interior of the pellets seeded at different spore levels after hard impact are presented in Table 3.

Table 3: SPORES RELEASED FROM THE INTERIOR OF INTERNALLY CONTAMINATED PELLETS AFTER IMPACTION

Velocity Ft/Sec	Mean Percent Spores Released	
	10^2(a)	10^5(a)
550	0.6	0.03
1500	0.4	0.30
3100	0.6	0.02
5100	0.2	0.002

(a) Initial Spore Level

It was noted that, at the four test velocities and the two inoculum levels, less than 1% of the available number of spores in the pellets were released. The apparent low values obtained for the pellets seeded at the 10^5 spore level may be at least partially explained by the counting method employed. The large number of organisms released necessitated early colony counting to avoid microbial overgrowth. Thus, the numbers observed and recorded may have been somewhat less than the number of spores actually released. A photograph of organisms released from a 550 ft/sec impacted pellet is presented in Figure 8.

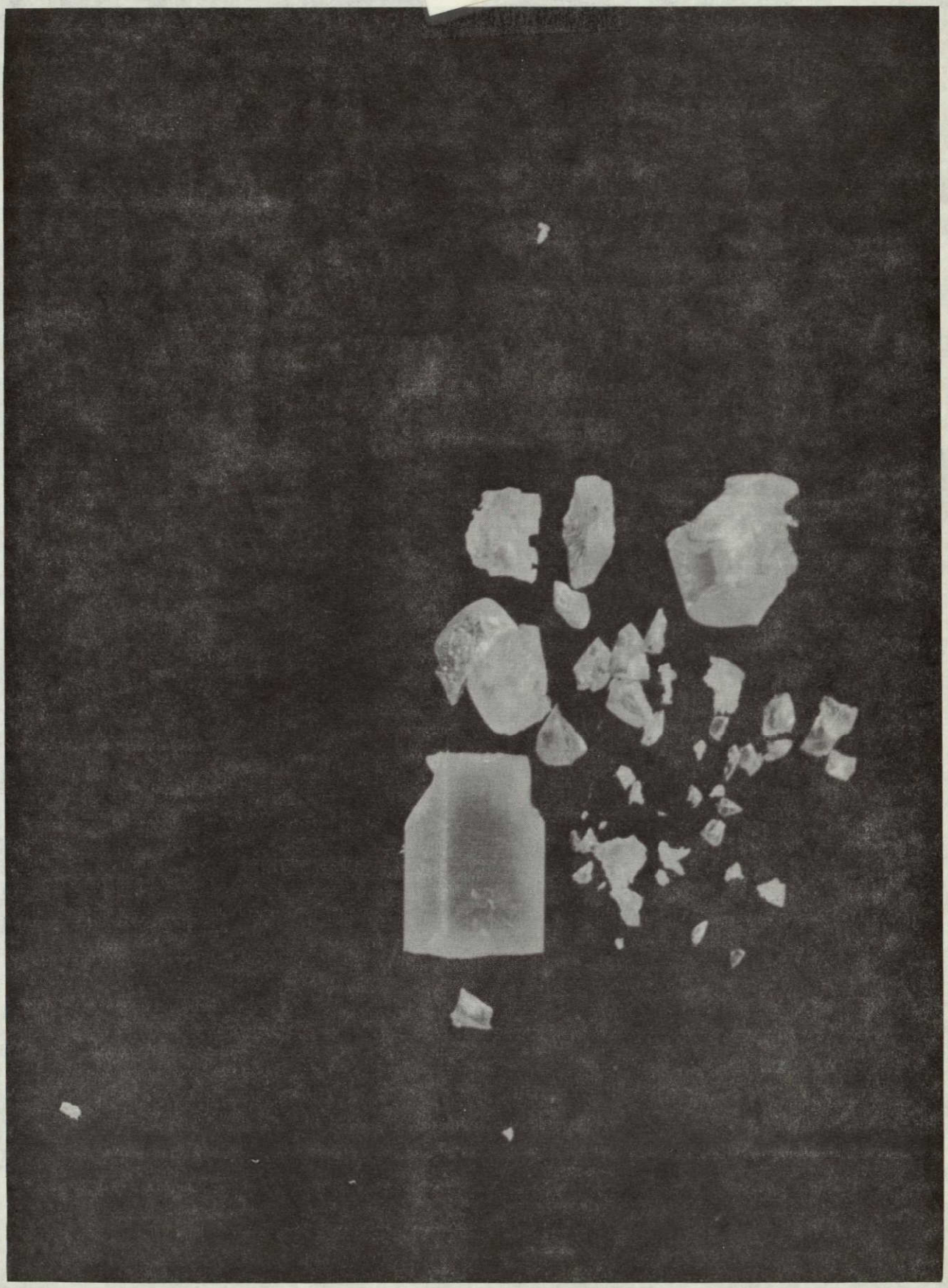

Figure 4: METHYL METHACRYLATE PELLET AFTER IMPACTION ON STAINLESS STEEL AT 550 FT/SEC

Figure 5: METHYL METHACRYLATE PELLET AFTER IMPACTION ON STAINLESS STEEL AT 1500 FT/SEC

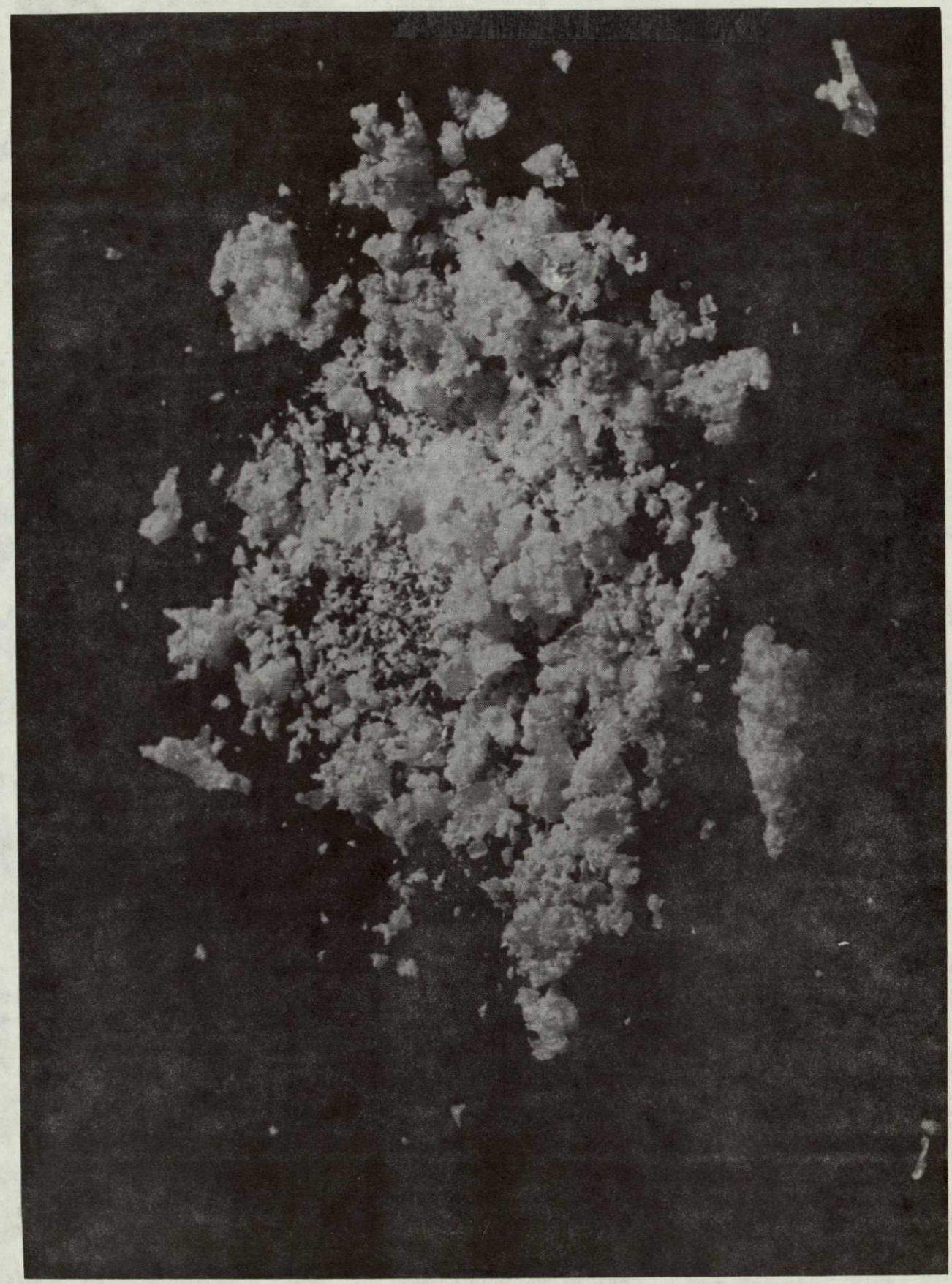

Figure 6: METHYL METHACRYLATE PELLET AFTER IMPACTION ON STAINLESS STEEL AT 3100 FT/SEC

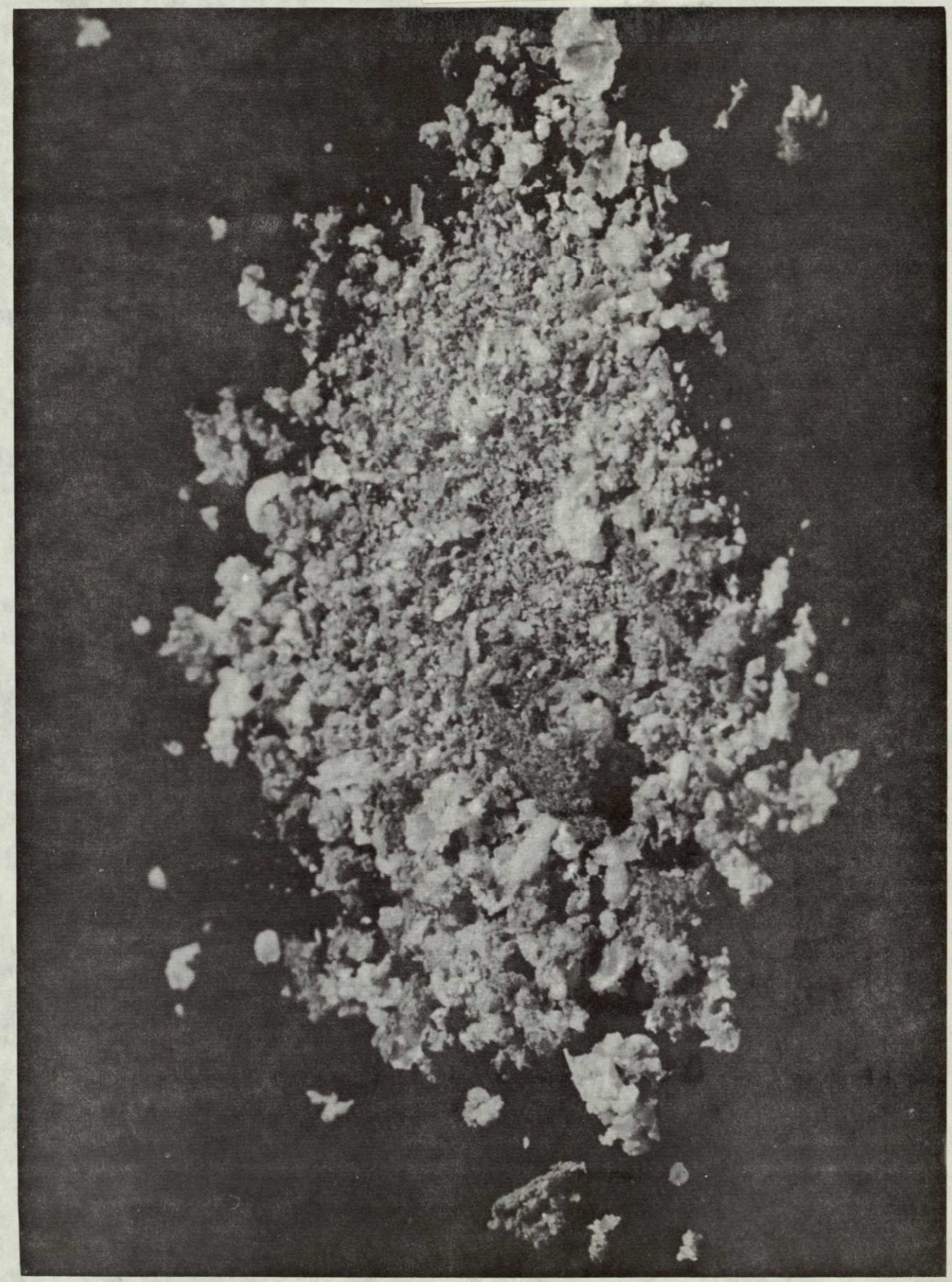

Figure 7: METHYL METHACRYLATE PELLET AFTER IMPACTION ON STAINLESS STEEL AT 5100 FT/SEC.

Figure 8: BACILLUS SUBTILIS VAR. NIGER COLONIES ON A METHYL METHACRYLATE PELLET PARTICLE AFTER IMPACTION ON STAINLESS STEEL AT 550 FT/SEC

4.3 EFFECT OF IMPACT ON SPORE SURVIVAL

The total number of viable spores recovered after hard impact at the four test velocities demonstrated that survival was dependent upon the velocity of impact. As the velocity was increased, fewer numbers of viable organisms were obtained (Table 4).

Table 4: EFFECT OF IMPACT UPON SURVIVAL OF SPORES IN THE INTERIOR OF METHYL METHACRYLATE

Velocity Ft/Sec	Mean Percent Spores Surviving Impact	
	10^2 (a)	10^5 (a)
550	63	82
1500	54	69
3100	5	9
5100	0.2	0.7

(a) Initial spore level

4.4 TEST CONTROLS

The surface sterilization method was found to eliminate all external spores from the projectiles. The procedural controls processed during Test IA and IB established that a background contamination level of B. subtilis var. niger spores averaged 0.5 spore for release pellets and 0.9 spore for survivor pellets. These constants were employed to adjust the data.

4.5 STATISTICAL ANALYSIS

An appropriate analysis of variance technique was used to investigate the data obtained on spore release. The results of this analysis indicate that there is not a significant difference ($F = 0.75$ with 3 and 40 degrees of freedom) in the percentage of spores released at the four velocities tested. The differences were examined at the 5% level.

A second analysis of variance was conducted to investiage spore survival after impact. This analysis revealed a highly significant effect ($F = 70.9$ with 3 and 40 degrees of freedom) of velocity on the percentage of spore survival after impact.

An analysis of variance was also conducted to assess the difference in inoculum levels. It was shown that percent release differences were highly significant ($F = 7.89$ with 1 and 40 degrees of freedom) between the two inoculum levels of pellets. Significant percentage differences ($F = 4.59$ with 1 and 40 degrees of freedom) were also demonstrated between the inoculum levels on the survival data.

5.0 SUMMARY

Internally contaminated methyl methacrylate pellets containing 10^2 or 10^5 B. subtilis var. niger spores were impacted upon stainless steel at velocities of 550, 1500, 3100 and 5100 ft/sec. After impact, the pellet particles were analyzed to determine: (1) the percentage of spores that were released from the pellet interior, or (2) the effect of impact upon the percentage survival of all the spores in the pellet.

Statistical analysis revealed that the effect of velocity was not significant for the percentage of spores released at impact. An average of the release data at all velocities show the mean percentage of organisms released to be 0.43 spore for the pellets seeded with 10^2 spores and 0.09 for the 10^5 level pellets. Therefore, the results show that less than 1% of the available number of spores will be released on impact. The total spore survival after impact data show consistent decreases in the number of viable spores recovered as the velocity of impact increases. In order to fully evaluate Test I data it should be combined with data from the total program. This has been done in Section I of this report.

6.0 APPENDIX

6.1 MATERIALS AND EQUIPMENT

6.1.1 Methyl Methacrylate (Eastman Chemical)

Methyl methacrylate powder (P-4942) and methyl methacrylate liquid (8334) were used to fabricate the plastic pellets. The preservative present in the liquid methyl methacrylate was removed by the washing process prior to pellet fabrication as outlined in the text.

6.1.2 Sodium Hydroxide

A 2% solution of sodium hydroxide in distilled water was prepared and stored in a screw-capped bottle. The sodium hydroxide was used to remove the preservative from the liquid methyl methacrylate.

6.1.3 Chlorine

A dilution of Chlorox bleach in distilled water was prepared to effect a final solution of 2,000 ppm chlorine and this solution was mixed fresh when needed. The solution was used to surface sterilize the projectiles.

6.1.4 Thiosulfate Solution

A 2,000 ppm solution of sodium thiosulfate in distilled water was prepared, Millipore membrane filter sterilized and stored in a sterile screw-capped bottle. It was used the same day it was prepared. The solution was employed to neutralize any residual chlorine on the pellet surface.

6.1.5 Acetone

Reagent grade Baker acetone was filter sterilized through a Nuclepore membrane and collected in a sterile side arm flask. The sterile acetone was transferred aseptically to dry, sterile one liter screw-capped bottles and stored until used. The sterile acetone was used to dissolve solid pellets and pellet particles.

6.1.6 Nuclepore Membranes (General Electric)

Nuclepore membranes 0.47 mm diameter, 0.5 μ pore size filters were wrapped in Kraft paper and autoclaved for 20 minutes at 121°C. Two filters were selected at random from each package and plated in TSA to monitor for sterility. The Nuclepore filters were used to recover spores from acetone.

6.1.7 Millipore Membranes (Millipore Corporation Corporation Bedford, Mass.)

Millipore membranes 0.47 mm diameter, 0.45 μ pore size were autoclaved for 20 minutes at 121°C. Two filters were selected at random from each package and plated in TSA to monitor for sterility. The Millipore filters were used to recover spores from peptone water canister washings.

6.1.8 Trypticase Soy Agar (Baltimore Biological Laboratories)

Twenty grams of Trypticase Soy Agar (TSA) were placed in a one liter screw capped bottle containing 500 mls distilled water. The bottle was labelled with autoclave tape and sterilized. Twenty-four bottles of TSA were autoclaved for 30 minutes at 121°C. After the sterilization cycle, the TSA was stored in a 50°C dry heat oven. TSA was used as the nutrient medium for the enumeration of viable spores.

6.1.9 Peptone Water (Difco)

Peptone was added to distilled water to effect a final concentration of 0.1%. Twenty-four one liter screw-capped bottles were filled with 800 mls of peptone water, labelled with autoclave tape and autoclaved for 30 minutes at 121°C. After sterilization, the bottles were allowed to cool to room temperature and stored in a cabinet until used.

6.1.10 Ultrasonic Bath (Delta Sonics)

An ultrasonic generator, Model DS-825, and an ultrasonic tank, Model NT-17(12) was employed in the test program. The equipment has a power output of 850 watts average, and 1700 watts peak. The output frequency is rated at 25 KC ± 3 KC. The lower power setting delivers 60% maximum power and high power delivers maximum power.

6.1.11 Autoclave

A Wilmot Castle Thermatic 60, automatic autoclave was available for the sterilization of materials used in the test program.

6.1.12 Miscellaneous Laboratory Equipment

All glassware, rubber stoppers, Millipore holders, forceps, magnetic stirring bars, collection canisters, and other laboratory equipment that was used in the study were autoclaved at 121°C for a sufficient period to render the objects sterile. Materials that were unable to withstand excessive heat were either sterilized with ethylene oxide gas or by surface chemical treatment. In addition, glassware was dried after sterilizing by holding the object in a dry heat oven until observable moisture disappeared.

6.2 UNREDUCED PROGRAM DATA

The unreduced data for Test I is presented in Tables 5 through 11.

Table 5 : PERCENT RECOVERY OF PELLET MATERIAL FOLLOWING IMPACT ON STAINLESS STEEL

Pellet Number	Velocity Ft/Sec	Weight Before Firing, G.	Weight After Impact, G.	% Recovered
1	531	0.938	0.936	99.8
2	714	0.924	0.911	98.6
3	683	0.933	0.930	99.7
4	654	0.931	0.922	99.0
5	723	0.937	0.931	99.4
6	472	0.937	0.936	99.9
7	1,530	0.938	0.838	89.3
8	1,820	0.940	0.911	96.9
9	1,740	0.933	0.898	96.2
10	1,840	0.939	0.865	92.1
11	1,250	0.937	0.856	91.4
12	1,560	0.934	0.890	95.3
13	3,040	0.940	0.813	86.5
14	3,230	0.938	0.803	85.6
15	3,180	0.938	0.826	88.1
16	2,900	0.930	0.863	92.8
17	3,210	0.940	0.792	84.3
18	3,280	0.937	0.782	83.5
19	4,950	0.941	0.637	67.7
20	4,900	0.934	0.648	69.4
21	5,275	0.929	0.577	62.1
22	5,060	0.926	0.604	65.2
23	5,260	0.936	0.622	66.5

Table 6 : TOTAL SPORE LEVEL OF CONTROL PELLETS OF TEST IA

Pellet Number	Pellet Weight, Gram	Spore Count	Spore Count/ Gram Pellet	Total Spore Level/ Gram Pellet (a)	Plastic Batch
IA-1-23	0.860	129	150.0	172.9	IA-1
IA-1-22	0.862	185	214.6		
IA-1-21	0.864	171	197.9		
IA-1-20	0.869	112	128.9		
IA-2-23	0.928	93	100.2	100.8	IA-2
IA-2-22	0.919	170	185.0		
IA-2-21	0.929	47	50.6		
IA-2-20	0.935	63	67.4		
IA-3-23	0.925	161	174.1	206.1	IA-3
IA-3-22	0.939	162	172.5		
IA-3-20	0.940	170	180.9		
IA-3-21	0.936	278	297.0		
IA-4-24	0.896	161	179.7	176.9	IA-4
IA-4-23	0.906	162	178.8		
IA-4-22	0.929	155	166.8		
IA-4-21	0.933	170	182.2		
IA-6-24	0.902	177	196.6	175.4	IA-6
IA-6-23	0.915	142	155.2		
IA-6-22	0.894	144	161.1		
IA-6-21	0.891	168	188.6		

(a) Mean for that plastic batch

Table 7 : TOTAL SPORE LEVEL OF CONTROL PELLETS OF TEST IB

Pellet Number	Pellet Weight, Grams	Spore Count	Spore Count/ Gram Pellet	Total Spore Level/Gram Pellet (a)	Plastic Batch
IB-1-35	0.852	320,000	375,587		
IB-1-34	0.864	510,000	590,278	578,303	IB-1
IB-1-27	0.880	550,000	625,000		
IB-1-26	0.886	640,000	722,348		
IB-2-33	0.852	560,000	657,277		
IB-2-32	0.843	560,000	664,294	565,487	IB-2
IB-2-22	0.937	350,000	363,583		
IB-2-21	0.935	530,000	566,845		
IB-3-24	0.897	230,000	256,410		
IB-3-23	0.914	280,000	306,346	387,079	IB-3
IB-3-20	0.934	440,000	471,092		
IB-3-19	0.933	480,000	514,470		
IB-4-25	0.840	302,000	359,524	395,269	IB-4
IB-4-24	0.877	378,000	431,015		
IB-5-18	0.924	550,000	595,238		
IB-5-16	0.934	500,000	535,332	551,172	IB-5
IB-5-12	0.937	490,000	522,946		
IB-6-10	0.888	380,000	427,928	491,742	IB-6
IB-6-9	0.936	520,000	555,556		

(a) Mean for that plastic batch

Table 8 : SPORES RELEASED FROM INTERNALLY CONTAMINATED METHYL METHACRYLATE AFTER IMPACT ON STAINLESS STEEL - TEST IA

Velocity Ft/Sec	Pellet Number	Pellet Weight, Grams	Initial Spore Level of Plastic Batch, G. Pellet	Spores Released from Pellet at Impact	Spores Released from G. Pellet at Impact (a)	Percent Spores Released
578	IA-4-7	0.937	177	1	0.6	0.3
579	IA-4-8	0.942	177	0	0	0
568	IA-4-9	0.939	177	2	1.7	0.9
548	IA-4-10	0.941	177	2	1.7	0.9
529	IA-4-11	0.943	177	2	1.7	0.9
579	IA-4-12	0.941	177	1	0.6	0.3
1575	IA-3-13	0.941	206	1	0.6	0.3
1540	IA-3-14	0.940	206	0	0	0
1655	IA-3-15	0.941	206	2	1.7	0.8
1585	IA-3-16	0.939	206	0	0	0
1645	IA-3-17	0.937	206	0	0	0
1560	IA-1-14	0.921	173	2	1.7	1.0
3210	IA-1-1	0.941	173	2	1.7	1.0
3110	IA-1-2	0.939	173	0	0	0
3120	IA-1-3	0.941	173	4	3.8	2.2
3140	IA-1-4	0.941	173	0	0	0
3390	IA-1-5	0.941	173	0	0	0
3090	IA-1-6	0.941	173	1	0.6	0.3
4930	IA-2-1	0.939	101	1	0.6	0.6
5180	IA-2-3	0.939	101	0	0	0
5380	IA-2-7	0.939	101	0	0	0
5200	IA-2-8	0.940	101	0	0	0
5260	IA-2-9	0.938	101	0	0	0
5250	IA-2-10	0.940	101	1	0.6	0.6

(a) Background contamination level established at 0.5 spore per pellet; corrected values.

Table 9 : SPORES RELEASED FROM INTERNALLY CONTAMINATED METHYL METHACRYLATE AFTER IMPACT ON STAINLESS STEEL - TEST IB

Velocity Ft/Sec.	Pellet Number	Pellet Weight, Grams	Initial Level Spores/G Pellet Before Impact	Number Spores Released Upon Impact	Number Spores Released/G Pellet Upon Impact (a)	Percent Spores Released
544	IB-1-1	0.934	578,303	64	68.1	0.01
568	IB-1-3	0.913	578,303	265	289.8	0.05
505	IB-1-4	0.931	578,303	27	28.5	0.01
569	IB-1-5	0.934	578,303	222	237.2	0.04
552	IB-1-6	0.920	578,303	401	435.4	0.08
564	IB-1-25	0.906	578,303	73	80.1	0.01
1520	IB-4-1	0.938	395,269	1052	1121.1	0.28
1570	IB-4-4	0.938	395,269	1017	1083.8	0.27
1445	IB-4-5	0.940	395,269	1328	1412.3	0.37
1490	IB-4-6	0.910	395,269	1304	1431.5	0.36
1535	IB-4-13	0.935	395,269	1089	1164.3	0.30
1535	IB-4-14	0.937	395,269	854	910.9	0.23
3230	IB-2-25	0.856	565,487	40	46.3	0.01
3350	IB-2-26	0.848	565,487	90	105.7	0.02
3000	IB-2-28	0.845	565,487	92	108.4	0.02
3240	IB-4-10	0.938	395,269	53	56.1	0.01
2880	IB-4-12	0.933	395,269	180	192.5	0.05
3230	IB-5-15	0.937	551,172	95	100.9	0.02
5260	IB-3-1	0.939	387,079	6	5.9	0.002
5030	IB-3-3	0.936	387,079	3	2.7	0.001
5210	IB-3-7	0.937	387,079	13	13.4	0.004
5050	IB-3-4	0.937	387,079	8	8.1	0.002
5100	IB-3-5	0.937	387,079	12	12.3	0.003
5160	IB-3-6	0.937	387.079	2	1.7	0.004

(a) Background contamination established to be 0.5 spore per pellet, corrected values.

Table 10 : EFFECT OF IMPACT ON STAINLESS STEEL UPON THE SUBSEQUENT SURVIVAL OF SPORES IN THE INTERIOR OF METHYL METHACRYLATE PELLETS - TEST IA

Velocity Ft/Sec	Pellet Number	Pellet Weight, Grams	Initial Spore Level of Plastic Batch, G. Pellet	Spores Surviving Impact	Spores, Surviving Impact/G Pellet (a)	Percent Spores Surviving Impact
574	IA-6-1	0.938	206	128	129.1	73.6
597	IA-6-2	0.935	206	120	66.4	37.9
542	IA-6-3	0.939	206	74	191.8	109.4
573	IA-6-4	0.939	206	91	99.2	56.6
565	IA-6-5	0.940	206	148	74.6	42.5
548	IA-6-6	0.940	206	91	99.1	56.5
1500	IA-4-1	0.940	177	89	93.7	53.0
1380	IA-4-2	0.932	177	127	135.3	76.5
1550	IA-4-3	0.941	177	111	117.0	66.2
1565	IA-4-4	0.939	177	42	43.8	24.8
1620	IA-4-6	0.942	177	57	59.6	33.7
1585	IA-1-13	0.935	173	118	125.3	72.5
3120	IA-1-7	0.941	173	3	2.2	1.3
3150	IA-1-8	0.942	173	10	9.7	5.6
3150	IA-1-9	0.943	173	0	0	0
3180	IA-1-10	0.942	173	0	0	0
3000	IA-1-11	0.942	173	20	20.3	11.7
3090	IA-1-12	0.939	173	17	17.2	9.9
5230	IA-2-5	0.939	101	2	1.2	1.2
5080	IA-2-6	0.938	101	0	0	0
5250	IA-2-11	0.937	101	0	0	0
5100	IA-2-12	0.940	101	0	0	0
5180	IA-2-15	0.940	101	0	0	0
4960	IA-4-14	0.936	177	1	0.1	0.1

(a) Background contamination level established at 0.9 spores per pellet.

Table 11 : EFFECT OF IMPACT ON STAINLESS STEEL UPON THE SUBSEQUENT SURVIVAL OF SPORES IN THE INTERIOR OF METHYL METHACRYLATE PELLETS - TEST IB

Velocity Ft/Sec	Pellet Number	Pellet Weight, Grams	Initial Level Spores/G Pellet Before Impact	Number Spores Surviving Impact	Number Spores Surviving Impact/G Pellet	% Spore Surviving Impact
539	IB-1-7	0.932	578,303	432,063	463,586	80.2
580	IB-1-8	0.936	578,303	495,300	529,166	91.5
574	IB-1-9	0.879	578,303	430,950	490,272	84.8
567	IB-1-10	0.934	578,303	518,700	555,352	96.0
590	IB-2-13	0.934	565,487	397,800	425,910	75.3
563	IB-2-14	0.936	565,487	347,600	371,368	65.7
1400	IB-5-7	0.937	551,172	355,500	379,402	68.8
1560	IB-5-8	0.934	551,172	332,000	355,460	64.5
1600	IB-5-9	0.937	551,172	164,000	175,026	31.8
1500	IB-5-10	0.939	551,172	483,600	515,015	93.4
1570	IB-5-11	0.937	551,172	425,000	453,574	82.3
1540	IB-6-2	0.938	491,742	328,000	349,679	71.1
3460	IB-2-29	0.874	565,487	24,413	27,932	4.9
3320	IB-2-30	0.824	565,487	26,465	32,117	5.7
3350	IB-2-11	0.891	565,487	40,300	45,229	8.0
3250	IB-2-12	0.938	565,487	49,140	52,387	9.3
3310	IB-2-27	0.833	565,487	31,590	37,922	6.7
3060	IB-4-21	0.841	395,269	70,800	84,185	21.3
5050	IB-3-15	0.934	387,079	5,948	6,367	1.6
5080	IB-3-8	0.935	387,079	5,986	6,401	1.7
5230	IB-3-9	0.937	387,079	609	649	0.2
5340	IB-3-14	0.934	387,079	666	712	0.2
5320	IB-3-16	0.938	387,079	203	216	0.1
5340	IB-3-13	0.937	387,079	1,162	1,239	0.3

SECTION

SECTION III

TEST II

RELEASE OF MICROORGANISMS FROM METHYL METHACRYLATE IMPACTED ONTO SAND

TEST II

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TEST II

RELEASE OF MICROORGANISMS FROM METHYL METHACRYLATE IMPACTED INTO SAND

1.0 PURPOSE

An investigation was conducted to determine the percentage release of microorganisms embedded in pellets that were impacted in sand.

2.0 INTRODUCTION

Methyl methacrylate pellets were fabricated so that each pellet interior contained approximately 10^4 Bacillus subtilis var. niger spores. A series of pellets were propelled from a gun at 550, 1500, 3100 or 5100 feet per second, respectively. Impact occurred in sand. After sand impact, the pellet fragments were analyzed to determine the number of exposed viable microorganisms and the effect of impact upon the survival of the spores in the interior of the particles. The test program is presented in Table 1.

3.0 PROCEDURES

3.1 PELLET MANUFACTURE

3.1.1 Preparation of Spore Stock

A 0.1 ml aqueous suspension of 10^9/ml of Bacillus subtilis var. niger spores was placed in the bottom of sterile planchets. The stock spore suspension was a minimum of 6 months old. The planchets were dried overnight by airflow from a Class 100 clean bench. When all the water had evaporated, 10 planchets were placed in a bottle containing 30 ml ethanol and insonated for 20 minutes. The level of the ultrasonic bath water was adjusted so that it was half way up the side of the bottle. After insonation, the 30 ml spore-ethyl alcohol suspension was pipetted into a sterile, capped bottle. All planchets were rinsed in fresh ethyl alcohol and the washings plus additional alcohol were combined with the 30 ml stock to total 100 ml. The stock inoculum was refrigerated until used. A plate count of the spore stock was performed 1, 3, and 7 days after preparation to accurately determine the number of spores per ml.

Table 1: TEST PROGRAM - METHYL METHACRYLATE PELLETS IMPACTED ON SAND

Velocity Feet/Second	Released Count After Impact 10,000 Spores/G	Total Viable Spores After Impact 10,000 Spores/G	Procedural Control	Total Percent Pellet Recovery	Initial Spore Level (Not fired) 10,000 Spores/G
550	6 [a]	6	3	6	3
1500	6	6	3	6	3
3100	6	6	3	6	3
5100	6	6	3	6	3
	24	24	12	24	12

Total: 96 pellets

a = Number of Replicates

3.1.2 Inoculation of Methyl Methacrylate Powder

Forty grams of methyl methacrylate powder were placed in a petri plate. The desired number of spores plus enough ethyl alcohol to bring the volume to a total 40 ml per 40 grams of methyl methacrylate powder was added to the powder. The mixture was then manually stirred with a wooden applicator stick. The wet seeded powder was air dried in a Class 100 clean bench overnight. The dried seeded powder was then sifted through a 40 mesh screen and stored in a glass beaker. All seeded powder was used within one week of preparation.

3.1.3 Removal of Preservative from Liquid Methyl Methacrylate

One hundred ml of liquid methyl methacrylate was placed in a clean 250 ml separatory funnel. A 100 ml freshly prepared 2% solution of sodium hydroxide was then added. The mixture was gently swirled for one minute and allowed to separate into two fractions. The bottom pink fraction containing the inhibiter was discarded. Another 100 ml of 2% sodium hydroxide was added and the washing process repeated until no pink color was observed in the bottom layer. When the bottom layer was clear, one additional washing with 100 ml sodium hydroxide was performed. The washed liquid methyl methacrylate was rinsed with separate 100 ml volumes of distilled water to remove all traces of sodium hydroxide. The number of rinses was one more than the sodium hydroxide washings. All water (bottom layer) was drained from the funnel. The washed liquid methyl methacrylate was used within 24 hours of preparation.

3.1.4 Plastic Fabrication

Forty ml of washed liquid methyl methacrylate was added to 40 gm of seeded powder in a glass beaker and mixed with a wooden applicator stick until a uniform slurry was obtained. The mixture was poured into glass test tubes (13 x 100 mm) so that they were 2/3 full. After each tube was filled, the beaker was swirled to assure a homogeneous mixture. When all tubes were filled, they were immediately placed in a desiccator jar and the pressure reduced to 5 inches of mercury for 10 minutes. The vacuum was sufficient to create a slow bubbling action of the mixture in the tubes, but not so low as to

cause the mixture to "climb" out the tube. The tubes were transferred for curing to a 50°C water bath and heated for 1.5 hours. The water level of the bath was adjusted so that it was slightly above the plastic level in the tubes. After 1.5 hours the tubes were removed from the bath and allowed to cool at room temperature for 10 minutes. The plastic rods were removed by breaking the tubes. The rods were then stored in a glass jar in the freezer (-18°C) until they were machined into pellets.

3.1.5 Pellet Machining

A seeded methyl methacrylate rod was machined into firing pellets by turning the rod on a small bench lathe. Each finished pellet was approximately 0.32 inches in diameter, 0.64 inches long, and weighed approximately 1.0 gram. The machining sequence was to cut all rods of the same plastic batch to the established diameter. Face cuts were then made to finish the pellets to the correct length. The time necessary to machine the methyl methacrylate rods into finished projectiles did not exceed 8 hours for each plastic batch (40 grams powder plus 40 grams liquid).

3.1.6 Pellet Surface Sterilization

After machining, each pellet was identified by a number, weighed to the nearest one-hundredth of a gram and this information recorded. Each pellet was surface sterilized in a freshly prepared 2,000 ppm chlorine solution for 10 minutes. This was followed by a 10 minute soak in a fresh filter-sterilized 2% solution of sodium thiosulfate to neutralize the chlorine. Each pellet was stored in a freezer in a sterile, appropriately labelled, screw-capped test tube until launched. The maximum time a projectile was a room temperature, from weighing to final storage in the freezer, did not exceed 2 hours.

The standardization of the time that seeded methyl methacrylate was exposed to room temperature was established to avoid any large differences in spore levels for each pellet batch. Previous data indicated a one log reduction occurred in spore populations if seeded plastic was kept at room temperature for periods exceeding 3 days. Therefore, the total elapsed time from plastic fabrication, through pellet machining, weighing, surface sterilization to

final freezer storage did not exceed 24 hours at room temperature for each projectile.

3.2 PELLET LAUNCHINGS

3.2.1 Collection Canister

The sterile collection canister (Figure 1) was constructed such that an impacting pellet struck only sand. In order to facilitate recovery of the projectile particles and impacted sand, the impact area was isolated from the rest of the supporting sand by a rubber condom. With this apparatus, it was possible to work with a relatively small quantity of sand (40 gm) without changing the impact characteristics of the sand. A baffle was also incorporated in the collection device to aid in retaining the sand at impact.

3.2.2 Pellet Firings

The pellets were fired by personnel of the Damage Mechanics Laboratory. In order to assure reliability in the firings, one person was responsible for all launchings. The gun barrel was positioned so that impact occurred vertically into the sand in an upright canister (Figure 2). The gun was cleaned before each day's firing by first flushing the barrel and chamber with air followed by an ethanol scrub. The sterile collection canister containing the sand target was placed in the gun chamber in the support sand. The pellet was loaded in the breech using sterile forceps. The cotton plug in the canister was removed, the chamber door sealed, and the pellet launched. After firing, the chamber door was opened, the cotton plug replaced in the canister, the velocity recorded, and the canister returned to the Microbiology Laboratory for analysis.

The methyl methacrylate pellets were fired at velocities of 550 ($\pm$ 100), 1500 ($\pm$ 200), 3100 ($\pm$ 300) and 5100 ($\pm$ 300) ft/sec. Compressed air was employed to attain 550 ft/sec and the pellet fired into a chamber maintained at atmospheric pressure. A powder charge was used to launch pellets at 1500, 3100, or 5100 ft/sec. Target velocities were measured using breakscreen techniques.

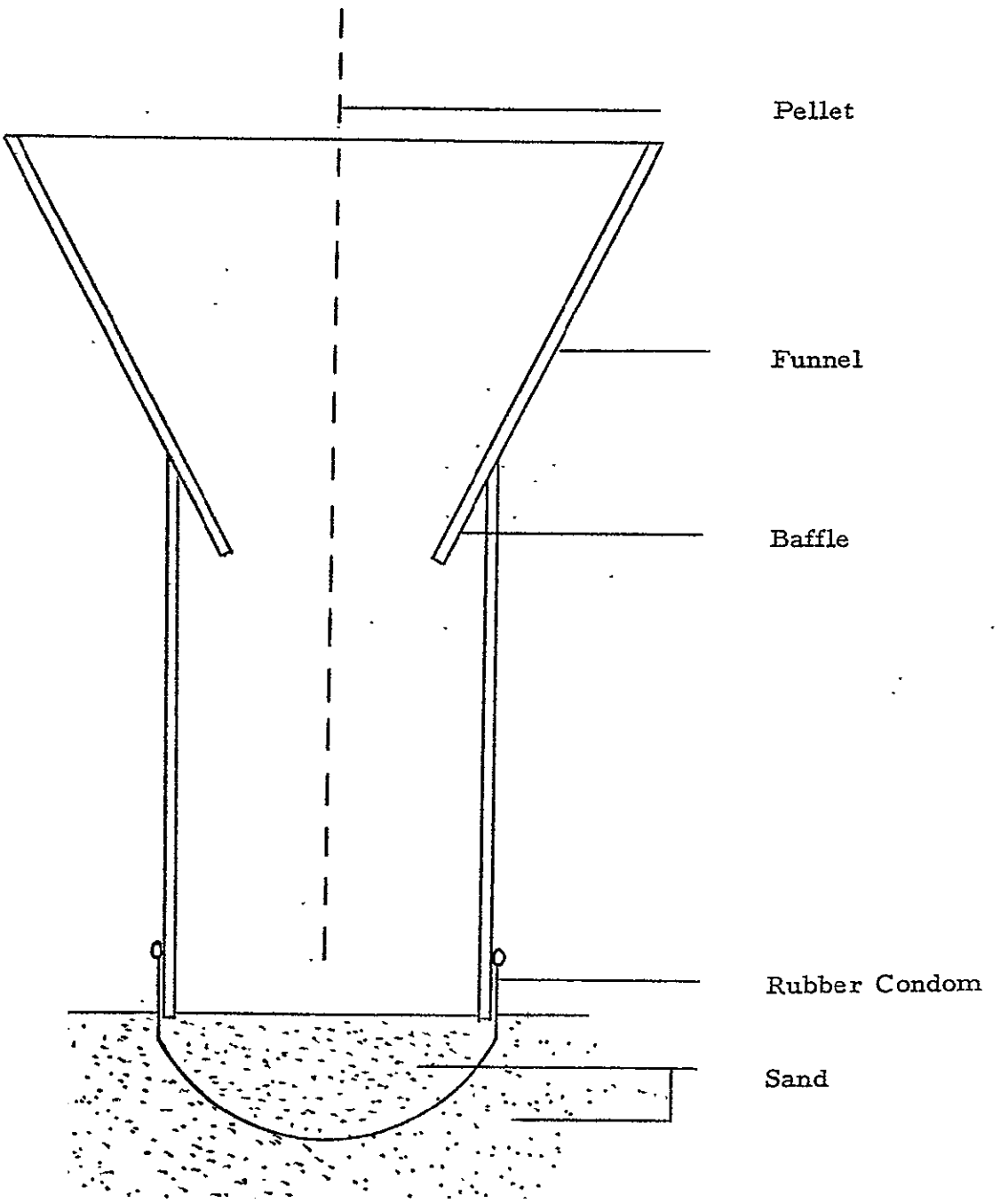

Figure 1: COLLECTION CANISTER FOR SAND IMPACT

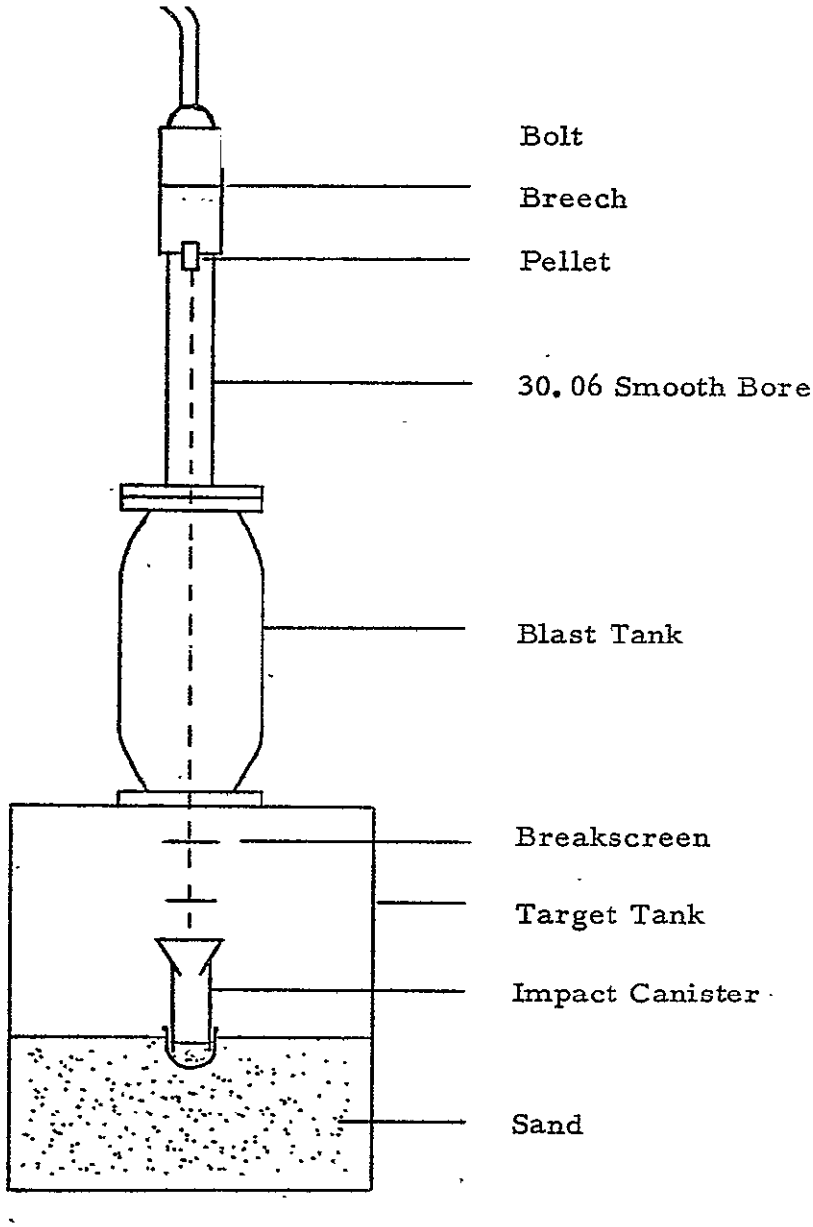

Figure 2: GAS AND POWDER GUN

A minimum of seven pellets were fired each day of Day 1, 2, 3, and 4 of a five day working week. Each day six seeded pellets were impacted in separate collection canisters. An analysis for spores released was conducted on three of the pellets while an analysis for total spore number surviving impact was performed on the remaining three pellets. An unseeded control pellet was fired and processed using the same analytical techniques. This last pellet launched each firing day established reliability of the test data with respect to possible contamination due to procedural techniques.

3.3 PELLET ANALYSIS

3.3.1 Percent Pellet Recovery After Impact

Six unseeded methyl methacrylate pellets were launched at each test velocity and impacted on sand. Each pellet was weighed, fired and collected in a separate canister. All particles of the impacted pellet were then weighed again. The procedures used to recover the plastic particles were identical to those that were employed in the analysis for released spores from seeded pellets after impact. The results of these firings established the percent of pellet recovered. The particles of each impacted pellet were saved for delivery to JPL.

3.3.2 Analysis for Spores Released After Pellet Impact

The collection canister, containing the impacted pellet, was analyzed in the Microbiology Laboratory. Analysis for the recovery of released spores was performed as outlined in Figure 3. The rubber condom containing the plastic particles and the impacted sand were removed from the canister. Large projectile particles were recovered by sifting the sand through a sterile screen mounted over a sterile breaker. The trapped pellet particles were transferred, with sterile forceps, to a sterile petri plate. Trypticase Soy Agar (TSA) was then poured into the plate so that each particle was completely covered with the medium. The sifted sand was poured into plates of molten TSA and gently swirled to insure even particle distribution. The sand and particles were incubated in TSA for 2 weeks at 30°C. Daily examinations were performed and each colony observed recorded as one released spore.

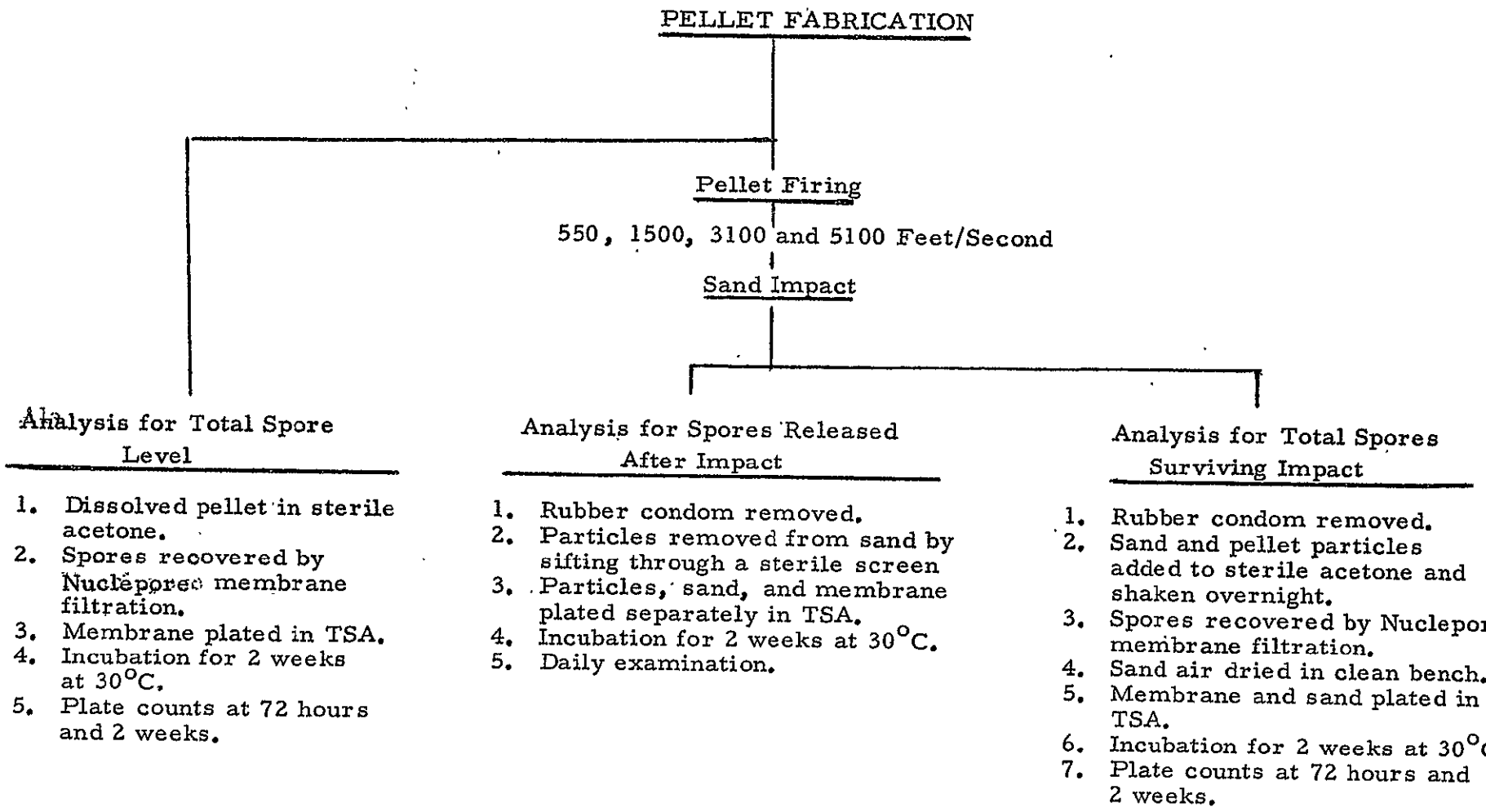

Figure 3: TEST ANALYSIS

3.3.3 Analysis for Total Number of Spores Surviving Impact

Analysis for the recovery of the total number of spores surviving impact was performed as outlined in Figure 3. The rubber condom containing the plastic particles and the impacted sand was removed from the canister. The contents of the rubber sheath were added to a one liter screw-capped bottle containing 400 ml sterile acetone and placed on the shaking platform in a 10°C refrigerated incubator. The bottle was shaken overnight to dissolve all particles. When complete dissolution occurred, the acetone was filtered through a Nuclepore filter to recover the spores. The filter was impinged upside down in molten TSA and incubated for 2 weeks at 30°C. The sand was poured into sterile 150 mm petri plates and dried in the airflow of a Class 100 clean bench. The dried sand was embedded in TSA and incubated for 2 weeks at 30 °C. At 72 hours, an initial colony count was made and additional observations made throughout the remainder of the incubation period. The spores counted were recorded as the total number of spores surviving impact.

3.3.4 Analysis for Total Spore Level

On each day of firing, two pellets were chosen at random, dissolved in acetone and the total spore level determined. Each pellet was placed in a one liter screw-capped bottle containing sterile acetone. The contents of the bottle were agitated overnight on a 10°C shaker. Filtration through a Nuclepore membrane recovered the spores from the acetone. The membrane was impinged upside down in molten TSA, incubated at 30°C for 2 weeks. This procedure established the number of spores initially present per gram of pellet and detected any reduction in the total spore number that might have occurred during storage.

3.3.5 Procedural Controls

On each test day, an unseeded control pellet was launched after the seeded pellets were fired. The control pellet particles were subjected to analysis as outlined in Figure 3. This control pellet established the reliability of the test data with respect to possible contamination due to procedural techniques. Also, on each test day, a seeded pellet was selected at random and embedded in melted TSA. The plate was incubated at 30°C and examined periodically

for 2 weeks. The absence of surface colonies established reliability in the method used to sterilize the pellet surface.

3.4 DATA RECORDING AND ANALYSIS

The data was recorded on data sheets that contained the date of pellet firing, pellet number and weight, the velocity of impact, the analysis performed and the results of the analysis. The data sheet for each test day was checked and initialed by the program manager.

These data provided information on:

(1) the number of viable organisms released from solids after hard impact;

(2) the percentage of viable microorganisms surviving impacts at the test velocities;

(3) differences in release and/or survival of organisms due to variations in impact velocities.

The data was evaluated statistically using analysis of variance techniques. These analyses detected any statistically significant difference between pellets in a replicate group and between pellets impacted at 550, 1500, 3100 and 5100 ft/sec.

4.0 RESULTS AND DISCUSSION

4.1 PERCENT PELLET RECOVERY AFTER IMPACT

The percentage of plastic recovered from the sand after pellet impaction at the four test velocities is presented in Table 2.

Table 2: PERCENT RECOVERY OF PELLET FOLLOWING IMPACT

Velocity Ft/Sec	Mean Percent Recovery of Pellet (a)
550	100
1500	100
3100	98
5100	100+

(a) Mean of 6 Pellets

The pellets fired at 550 and 1500 ft/sec did not fracture when they impacted sand. However, areas where sand abrasion had occurred were noted on some pellets. The higher velocities, 3100 and 5100 ft/sec, resulted in completely fractured pellets. Figures 4, 5, 6 and 7 show a comparison of the various types of pellet fracturing at the four test velocities.

4.2 SPORES RELEASED FROM THE INTERIOR OF IMPACTED PELLETS

The percentage of spores released from the interior of the pellets after sand impact are presented in Table 3.

Table 3: SPORES RELEASED FROM THE INTERIOR OF INTERNALLY CONTAMINATED PELLETS AFTER IMPACTION

Velocity Ft/Sec	Mean Percent Spores Released (a) (10^4 Spores Available for Release)
550	0.001
1500	0.001
3100	0.06
5100	0.06

(a) Mean of 6 Pellets

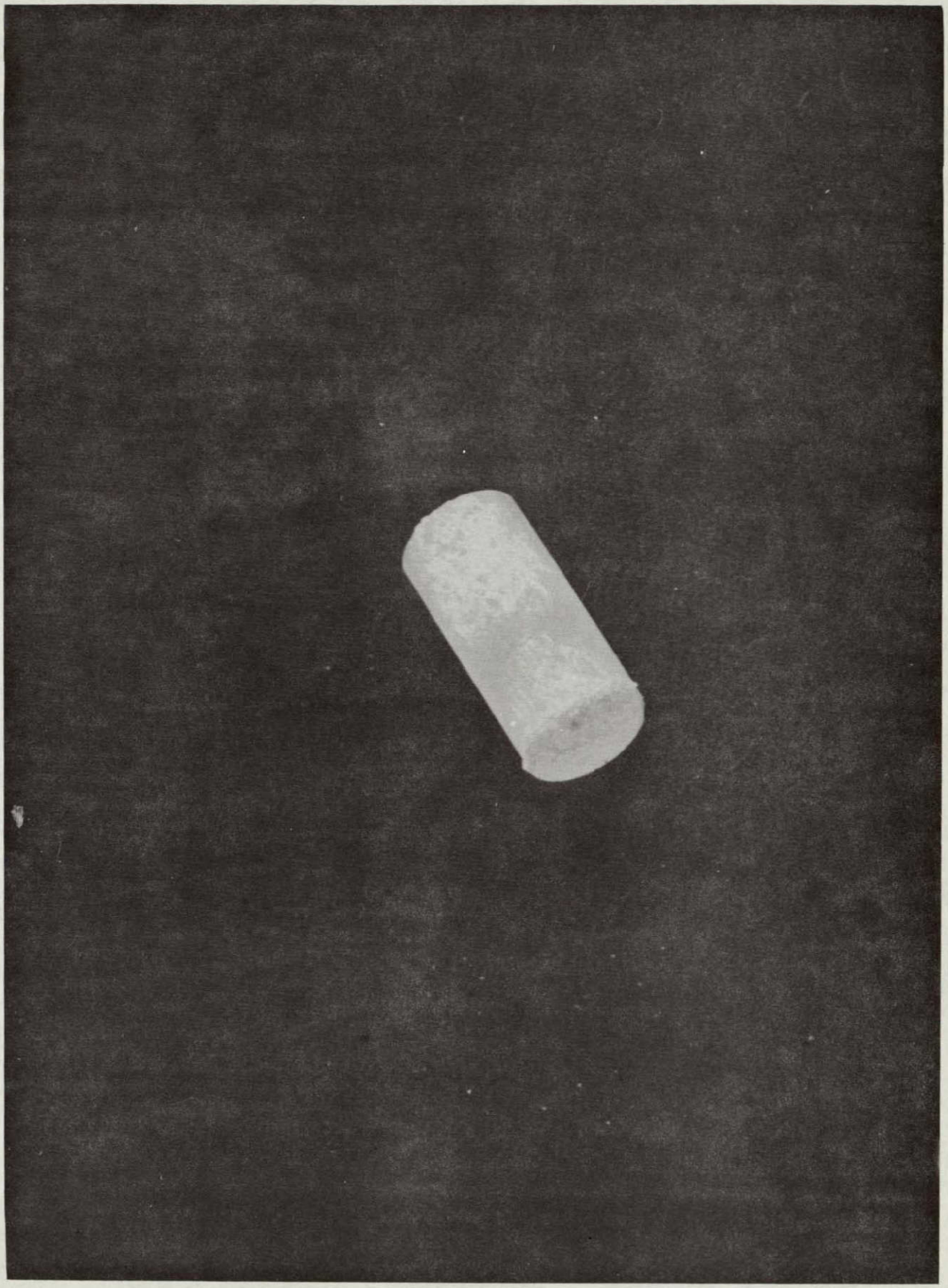

Figure 4: METHYL METHACRYLATE PELLET AFTER IMPACTION INTO SAND AT 550 FT/SEC

Figure 5: METHYL METHACRYLATE PELLET AFTER IMPACTION INTO SAND AT 1500 FT/SEC

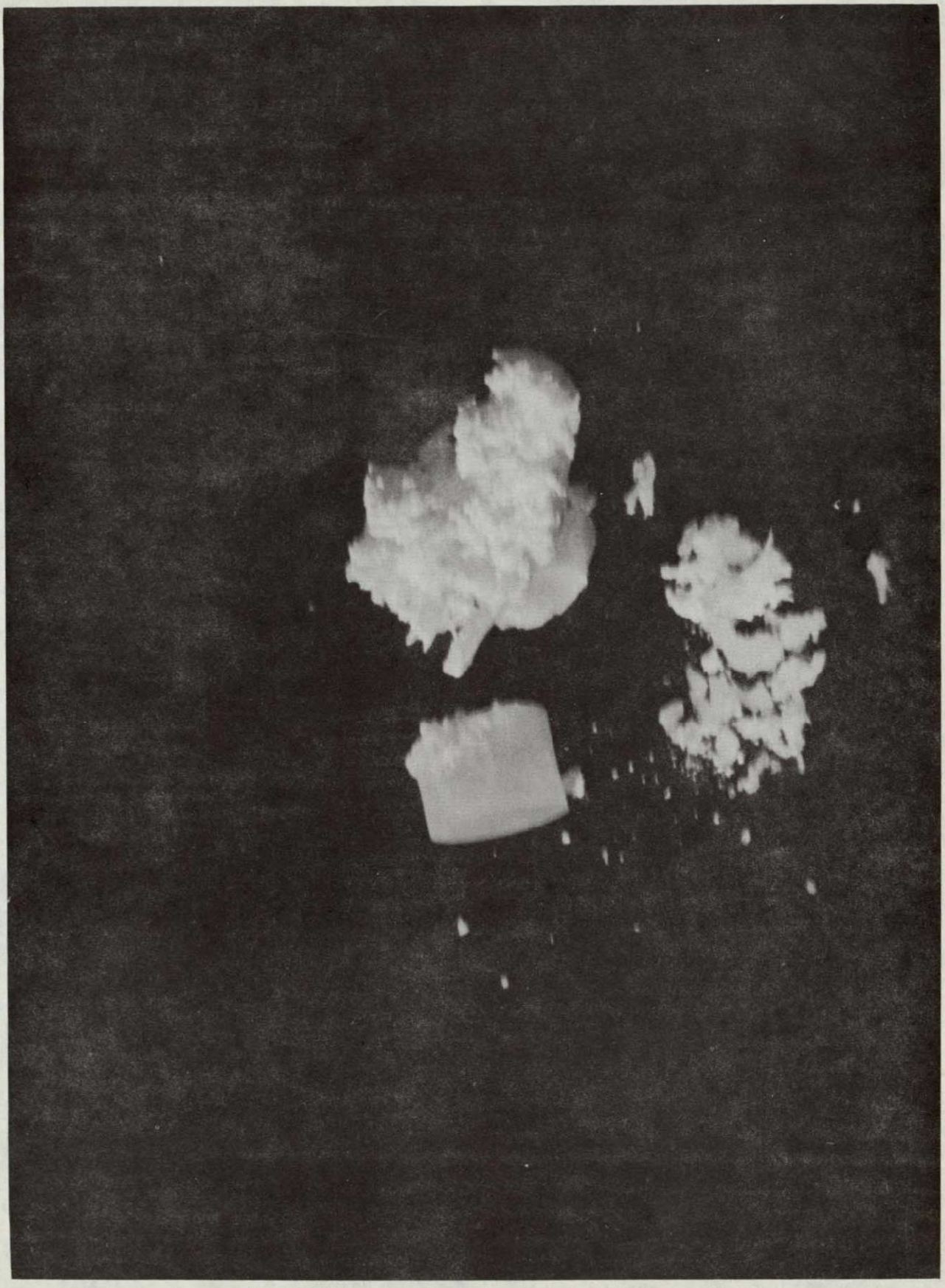

Figure 6: METHYL METHACRYLATE PELLET AFTER IMPACTION INTO SAND AT 3100 FT/SEC

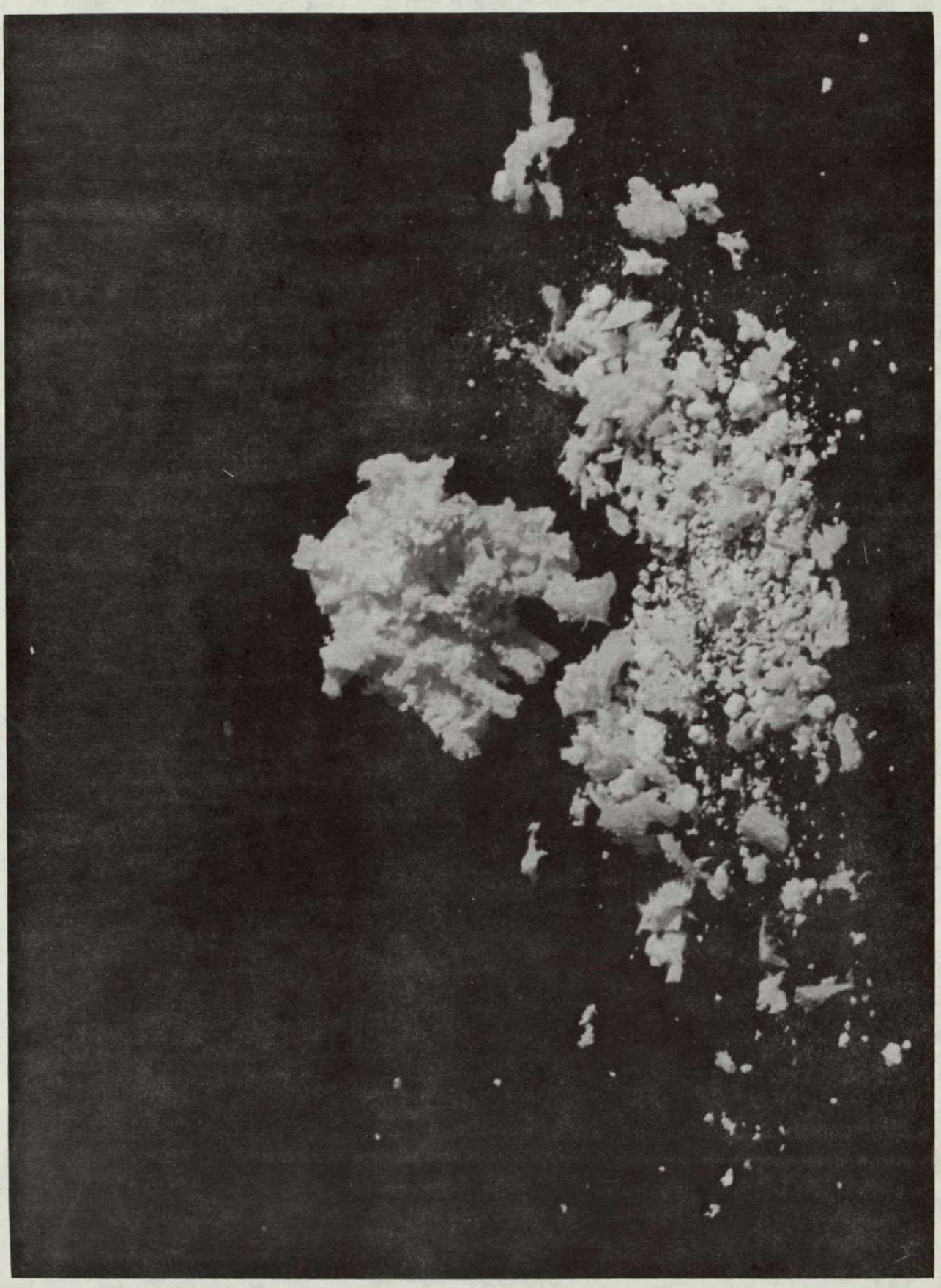

Figure 7: METHYL METHACRYLATE PELLET AFTER IMPACTION INTO SAND AT 5100 FT/SEC

It was noted that, at the four test velocities, less than one-tenth of one percent of the available number of spores in the pellets were released. For every 1000 organisms initially available, one or less spores were released when a pellet impacted on sand at all velocities.

4.3 EFFECT OF IMPACT ON SPORE SURVIVAL

The total number of viable spores recovered after sand impact at the four test velocities demonstrated that survival was dependent upon the velocity of impact. As the velocity was increased, fewer numbers of viable organisms were obtained (Table 4).

Table 4: EFFECT OF SAND IMPACT UPON THE SURVIVAL OF SPORES IN METHYL METHACRYLATE

Velocity Ft/Sec	Mean Percent of Spores Surviving Impact (a) (10^4 Spores Initially Present/Pellet)
550	100+
1500	98.8
3100	66.1
5100	18.5

(a) Mean of 6 pellets

4.4 TEST CONTROLS

No background contamination due to fabrication and handling of the plastic material, or due to the recovery and analytical procedures was observed. In addition, the surface sterilization method was found to eliminate all external spores from the projectiles.

4.5 STATISTICAL ANALYSIS

An appropriate analysis of variance method was used to investigate the data obtained on spore release. The results show no significant difference ($F = 2.87$ with 3 and 20 degrees of freedom) in the spore percentage release at the four velocities. The differences were examined at the 5% level. A second analysis of variance was conducted to investigate spore survival after impact. This analysis revealed a highly significant effect ($F = 57.3$ with 3 and 20 degrees of freedom) of velocity on the percentage of spore survival.

5.0 SUMMARY

This test was conducted to determine the number of microorganisms released from the interior of methyl methacrylate pellets impacted into sand. The pellets were fabricated so that each pellet interior contained approximately 10^4 B. subtilis var. niger spores. A series of pellets were propelled from a gun at 550, 1500, 3100 and 5100 ft/sec. Impact occurred in sand mounted in a collection canister. After impact, the pellet fragments were analyzed to determine the number of released viable microorganisms and to assess the effect of impact by determining the total numbers of surviving spores in the fragments from each pellet.

Analysis of variance of the percentage of spores released upon impact revealed no significant differences among the four test velocities. An average of less than 0.1 percent of the available organisms in the pellets were released by these impact.

The analysis also demonstrated that the total number of microorganisms surviving impact decreased as velocity increased. Survival ranged from 100 percent at 550 ft/sec to 18.5 percent at 5100 ft/sec. This decrease in survival with increased velocity was shown statistically to be highly significant. A discussion of various implications of these data are included in Section I of this report.

6.0 APPENDIX

6.1 MATERIALS AND EQUIPMENT

6.1.1 Methyl Methacrylate (Eastman Chemical)

Methyl methacrylate powder (P-4942) and methyl methacrylate liquid (8334) were used to fabricate the plastic pellets. The preservative present in the liquid methyl methacrylate was removed by the washing process prior to pellet fabrication as outlined in the text.

6.1.2 Sodium Hydroxide

A 2% solution of sodium hydroxide in distilled water was prepared and stored in a screw-capped bottle. The sodium hydroxide was used to remove the preservative from the liquid methyl methacrylate.

6.1.3 Chlorine

A dilution of Chlorox bleach in distilled water was prepared to effect a final solution of 2000 ppm chlorine. This solution was mixed fresh when needed. The solution was used to surface sterilize the projectiles.

6.1.4 Thiosulfate Solution

A 2% solution of sodium thiosulfate in distilled water was prepared, Millipore membrane filter sterilized, and stored in a sterile screw-capped bottle. It was used the same day it was prepared. The solution was employed to neutralize any residual chlorine on the pellet surface.

6.1.5 Acetone

Reagent grade acetone was filter-sterilized through Nuclepore membranes and collected in a sterile side arm flask. The sterile acetone was transferred asceptically to dry, sterile one liter screw-capped bottles and stored until used. The sterile acetone was used to dissolve solid pellets and pellet particles.

6.1.6 Nucleopore Membranes (General Electric)

Nucleopore membranes 0.47 mm diameter, 0.45 μ pore size filters were wrapped in Kraft paper and sterilized in an autoclave for 20 minutes at 121°C. Two filters were selected at random from each package and plated in TSA to monitor for sterility. The Nuclepore filters were used to recover spores from acetone.

6.1.7 Trypticase Soy Agar (Baltimore Biological Laboratories)

Twenty grams of Trypticase Soy Agar (TSA) were placed in a one liter screw-capped bottle containing 500 mls distilled water. The bottle was labelled with autoclave tape and sterilized. Twenty-four bottles of TSA were autoclaved for 30 minutes at 121°C. After the sterilization cycle, the TSA was stored in a 50°C dry heat oven. TSA was used as the nutrient medium for the enumeration of viable spores.

6.1.8 Peptone Water (Difco)

Peptone was added to distilled water to effect a final concentration of 0.1%. Twenty-four one liter screw-capped bottled were filled with 800 mls of peptone water, labelled with autoclave tape and autoclaved for 30 minutes at 121°C. After sterilization, the bottles were allowed to cool to room temperature and stored in a cabinet until used.

6.1.9 Ultrasonic Bath (Delta Sonics)

An ultrasonic generator, Model DS-825, and an ultrasonic tank, Model NT-17(12) were employed in the test program. The equipment has a power output of 850 watts average, and 1700 watts peak. The output frequency is rated at 25 KC $\pm$ 3 KC. The lower power setting delivers 60% maximum power and high power delivers maximum power.

6.1.10 Autoclave

A Wilmot Castle Thermatic 60, automatic autoclave was available for the sterilization of materials used in the test program.

6.1.11 Miscellaneous Laboratory Equipment

All glassware, rubber stoppers, membrane holders, forceps, magnetic stirring bars, collection canisters, and other laboratory equipment that was used in the study was autoclaved at 121°C for a sufficient period to render the objects sterile. Materials that were unable to withstand excessive heat were either sterilized with ethylene oxide gas or by surface chemical treatment. In addition, glassware was dried after sterilizing by holding the object in a dry heat oven until observable moisture disappeared.

6.2 UNREDUCED PROGRAM DATA

The unreduced data from Test II is presented in Tables 5 through 8.

Table 5 : PERCENT RECOVERY OF PELLET MATERIAL FOLLOWING SAND IMPACT

Pellet Number	Velocity Ft/Sec	Pellet Weight Before Impact, G	Pellet Weight After Impact, G	% Pellet Recovered
2A-1-1	550	0.941	0.936	99.5
2A-1-2	590	0.938	0.930	99.1
2A-1-3	527	0.940	0.935	99.5
2A-1-4	585	0.941	0.936	99.5
2A-1-5	587	0.940	0.935	99.5
34C	600	0.932	0.937	100.5
32C	1460	0.932	0.937	100.5
2A-2-7	1480	0.939	0.934	99.5
2A-2-9	1525	0.942	0.935	99.3
2A-2-6	1480	0.940	0.937	99.7
2A-2-5	1480	0.939	0.932	99.3
2A-2-4	1500	0.933	0.924	99.0
2A-3-5	3140	0.940	0.928	98.7
2A-3-8	3180	0.938	0.932	99.4
2A-3-6	3160	0.938	0.923	98.4
2A-3-4	3020	0.935	0.904	96.7
2A-3-15	3060	0.941		
2A-4-6	4930		0.962	
2A-1-17	5260	0.910	0.999	109.8
2A-1-15	4810	0.936	0.949	101.4
2A-1-14	4840	0.935	1.069	114.3
2A-1-13	5080	0.939	0.979	104.3

Table 6 : TOTAL SPORE LEVEL OF CONTROL PELLETS OF TEST II

Pellet Number	Pellet Weight, Grams	Spore Count	Spore Count/ Gram Pellet	Total Spore Level/Gram Pellet (a)	Plastic Batch
2A-1-24	0.930	10,650	11,452		
2A-1-23	0.924	11,250	12,175	10,296	2A-1
2A-1-22	0.914	7,200	7,878		
2A-1-21	0.930	9,000	9,677		
2A-2-24	0.884	10,500	11,878		
2A-2-23	0.897	8,400	9,365		
2A-2-22	0.926	8.250	8,909	10,659	2A-2
2A-2-21	0.865	10,800	12,486		
2A-3-24	0.938	8,700	9,275		
2A-3-23	0.937	8.700	9.285	10,051	2A-3
2A-3-22	0.936	11,100	11,859		
2A-3-21	0.935	9,150	9,786		
2A-4-21	0.886	7,200	8,126	7,757	2A-4
2A-4-20	0.934	6,900	7,388		
2A-5-24	0.880	8,100	9,205	8,532	2A-5
2A-5-23	0.878	6,900	7,859		

(a) Mean for that plastic batch

Table 7 : SPORES RELEASED FROM INTERNALLY CONTAMINATED METHYL METHACRYLATE AFTER IMPACT ON SAND - TEST II

Velocity Ft/Sec.	Pellet Number	Pellet Weight, Grams	Inital Level Spores/G Pellet Before Impact	Number Spores Released Upon Impact	Number Spores Released/G Pellet Upon Impact	Percent Spores Released
550	2A-1-1	0.941	10,296	0	0	0
590	2A-1-2	0.938	10,296	0	0	0
527	2A-1-3	0.940	10,296	0	0	0
585	2A-1-4	0.941	10,296	0	0	0
587	2A-1-5	0.940	10,296	0	0	0
590	2A-1-6	0.939	10,296	1	1.1	0.01
1500	2A-2-4	0.933	10,659	0	0	0
1480	2A-2-5	0.939	10,659	0	0	0
1480	2A-2-6	0.940	10,659	0	0	0
1480	2A-2-7	0.939	10,659	0	0	0
1560	2A-2-8	0.935	10,659	0	0	0
1525	2A-2-9	0.942	10,659	1	1.1	0.01
3200	2A-3-2	0.936	10,051	6	6.4	0.1
3020	2A-3-4	0.935	10,051	15	16.0	0.2
3140	2A-3-5	0.940	10,051	1	1.1	0.01
3160	2A-3-6	0.938	10,051	1	1.1	0.01
3180	2A-3-8	0.938	10,051	1	1.1	0.01
3060	2A-3-15	0.941	10,051	1	1.1	0.01
5080	2A-1-13	0.939	10,296	2	2.1	0.02
4840	2A-1-14	0.935	10,296	6	6.4	0.06
4810	2A-1-15	0.936	10,296	4	4.3	0.04
5260	2A-1-17	0.910	10,296	7	7.7	0.07
5260	2A-4-3	0.935	7,757	1	1.1	0.01
4930	2A-4-6	0.942	7,757	5	5.3	0.07

Table 8 : EFFECT OF IMPACT IN SAND UPON THE SUBSEQUENT SURVIVAL OF SPORES IN THE INTERIOR OF METHYL[E] METHACRYLATE PELLETS - TEST II

Velocity Ft/Sec.	Pellet Number	Pellet Weight, Grams	Initial Level Spores/ G Pellet Before Impact	Number Spores Surviving Impact	Number Spores/G Pellet Surviving Impact	Percent Spores Surviving
577	2A-1-7	0.937	10,296	13,050	13,927	135.3
553	2A-1-8	0.942	10,296	10,800	11,465	111.4
587	2A-1-9	0.940	10,296	12,300	13,085	127.1
490	2A-1-10	0.938	10,296	13,500	14,392	139.4
597	2A-1-11	0.941	10,296	15,450	16,419	159.5
540	2A-1-12	0.941	10,296	11,700	12,434	120.8
1510	2A-2-11	0.940	10,659	13,350	14,202	133.2
1460	2A-2-12	0.933	10,659	7,200	7,717	72.4
1450	2A-2-13	0.939	10,659	12,300	13,099	122.9
1480	2A-2-14	0.937	10,659	8,400	8,965	84.1
1530	2A-2-15	0.939	10,659	9,600	10,224	95.9
1420	2A-2-16	0.874	10,659	7,860	8,993	84.4
3100	2A-3-9	0.938	10,051	8,100	8,635	85.9
3050	2A-3-12	0.939	10,051	5,850	6,230	62.0
3160	2A-3-13	0.939	10,051	5,850	6,230	62.0
3220	2A-3-14	0.932	10,051	5,850	6,277	62.5
3120	2A-3-16	0.938	10,051	5,400	5,757	57.3
3150	2A-3-17	0.938	10,051	6,300	6,716	66.8
5110	2A-5-1	0.940	8,532	1,400	1,489	17.5
5130	2A-5-2	0.940	8,532	1,850	1,968	23.1
5200	2A-5-3	0.941	8,532	1,800	1,913	22.4
5180	2A-5-5	0.941	8,532	1,300	1,382	16.2
5210	2A-5-6	0.942	8,532	850	902	10.6
5070	2A-5-7	0.942	8,532	1,700	1,805	21.2

SECTION IV

TEST III

RELEASE OF MICROORGANISMS FROM ECCOBOND EPOXY AFTER HARD IMPACT ON STAINLESS STEEL

TEST III

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TEST III

RELEASE OF MICROORGANISMS FROM ECCOBOND EPOXY AFTER HARD IMPACT ON STAINLESS STEEL

1.0 PURPOSE

An investigation was conducted to determine the percentage release of microorganisms embedded in pellets that were impacted on stainless steel plates.

2.0 INTRODUCTION

Eccobond 55 pellets were fabricated so that each pellet interior contained approximately 10^3 Bacillus subtilis var. niger spores. A series of pellets were propelled from a gun at 550, 1500, 3100 or 5100 feet per second, respectively. Impact occurred on a stainless steel plate mounted in a collection canister. After impact, the pellet fragments were analyzed to determine the number of exposed viable microorganisms and the effect of impact upon the survival of the spores in the interior of the particles. The test program is presented in Table 1.

3.0 PROCEDURES

3.1 PELLET MANUFACTURE

3.1.2 Preparation of Spore Stock

A 0.1 ml aqueous suspension of Bacillus subtilis var. niger spores was placed in the bottom of sterile planchets. The stock spore suspension was a minimum of 6 months old. The planchets were dried overnight by airflow from a Class 100 clean bench. When all the water had evaporated, 10 planchets were placed in a bottle containing 30 ml ethanol and insonated for 20 minutes. The level of the bath water was adjusted so that it was half way up the side of the bottle. After insonation, the 30 ml spore-ethyl alcohol suspension was pipetted into a sterile, capped bottle. All planchets were rinsed in fresh alcohol and the washings plus additional alcohol were combined with the 30 ml stock to total 100 ml. The stock inoculum was refrigerated until used. A plate count of the spore stock was performed 1, 3 and 7 days after preparation to accurately determine the number of spores per ml.

Table 1: TEST III - ECCOBOND PELLETS IMPACTED ON STAINLESS STEEL PLATES

Velocity Ft/Sec	Released Count After Impact 1,000 Spores/G	Total Viable Spores After Impact 1,000 Spores/G	Procedural Controls	Total Percent Pellet Recovery	Initial Spore Level (not fired) 1,000 Spores/G
550	6 *	6	3	6	3
1500	6	6	3	6	3
3100	6	6	3	6	3
5100	6	6	3	6	3
	24	24	12	24	12

Total: 96 Pellets

* Number of Replicate Pellets

3.1.2 Epoxy Fabrication

Thirty-six grams of Eccobond 55 were placed in a glass beaker and 4.32 grams of Catalyst 9 added. A 6×10^6 spore inoculum in 0.2 ml of alcohol was added and the mixture thoroughly stirred. The glass beaker containing the liquid Eccobond was placed in a desiccator jar and the pressure reduced to 5 inches of mercury for 10 minutes. The mixture was removed from the jar and poured into 13 x 100 mm teflon tubes. The tubes were cured in a $50^{\circ}C$ oven for 3 hours.

After curing, the tubes were removed from the oven, allowed to cool at room temperature for 10 minutes, and the Eccobond removed. The Eccobond rods were stored in a glass jar in the freezer ($-18^{\circ}C$) until machining. All jars were labelled with information as to date of seeding, fabrication, batch number, and the number of spores per gram of epoxy.

3.1.3 Pellet Machining

A seeded Eccobond rod was machined into firing pellets by turning the rod on a small bench lathe. Each finished pellet was approximately 0.32 inches in diameter, 0.64 inches long, and weighed approximately 1.0 gram. The machining sequence was to cut all rods of the same epoxide batch to the established diameter. Face cuts were then made to finish the pellets to the correct length. The time necessary to machine the Eccobond rods into finished projectiles did not exceed 4 hours for each epoxy batch (36 grams of Eccobond 55 plus 4.32 grams #9).

3.1.4 Pellet Surface Sterilization

After machining, each pellet was identified by a number, weighed to the nearest one-hundredth of a gram and this information recorded. Each pellet was surface sterilized in a freshly prepared 2000 ppm chlorine solution for 10 minutes. This was followed by a 10 minute soak in a fresh filter-sterilized 2% solution of sodium thiosulfate. Each pellet was stored in a freezer in a sterile, appropriately labelled, screw-capped test tube until launched.

3.2 PELLET LAUNCHING

3.2.1 Collection Canister

A sterile collection canister (Figure 1) was constructed such that an impacting pellet struck the slanted stainless steel witness plate and all pellet particles remained trapped in the canister.

3.2.2 Pellet Firings

The pellets were fired by personnel of the Damage Mechanics Laboratory. In order to assure reliability in the firings, one person was responsible for all launchings. The gun (Figure 2) was cleaned before each day's firing by launching 2 sterile pellets to flush the barrel and chamber. The sterile collection canister containing the target of stainless steel was then positioned in the gun chamber. The pellets were loaded in the breech using sterile forceps. The cotton plug in the canister was removed, the chamber door sealed, and the pellet launched. After firing the chamber door was opened, the cotton plug replaced in the canister, the velocity recorded, and the canister returned to the Microbiology Laboratory for analysis.

The epoxy pellets were fired at velocities of 550 (± 100), 1500 (± 200), 3100 (± 300) and 5100 (± 300) ft/sec. Compressed air was employed to attain 550 ft/sec. The pellet was fired into a chamber maintained at atmospheric pressure. A powder charge was used to launch the pellets at 1500, 3100, or 5100 ft/sec. Pellet velocities were measured using breakscreen techniques.

A minimum of seven pellets were fired each day of Day 1, 2, 3, and 4 of a five day working week. Each day six seeded pellets were impacted in separate collection canisters. An analysis for spores released was conducted on three of the pellets and an analysis for total spore number surviving impact was performed on the remaining three pellets. An unseeded control pellet was fired and processed using the same techniques. This control pellet established the reliability of the test data with respect to possible contamination due to procedural techniques.

3.3 PELLET ANALYSIS

3.3.1 Percent Pellet Recovery After Impact

Six unseeded Eccobond pellets were launched at each test velocity and impacted on stainless steel. Each pellet was weighed, fired, and collected in a separate

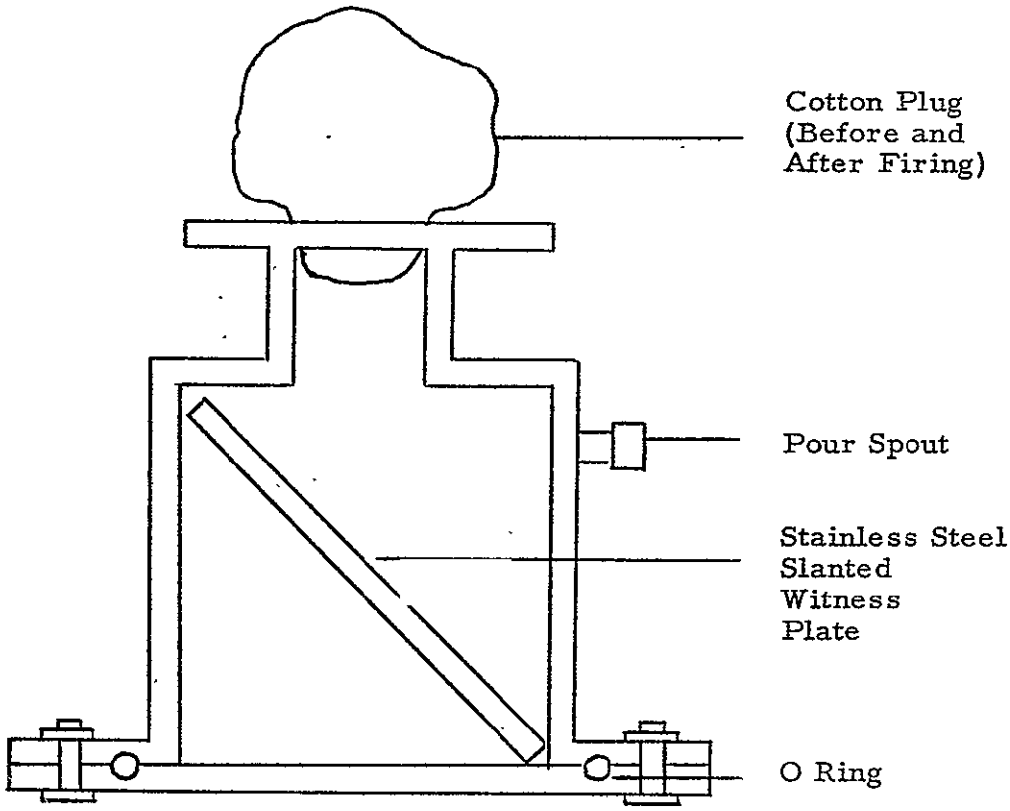

Figure 1: COLLECTION CANISTER

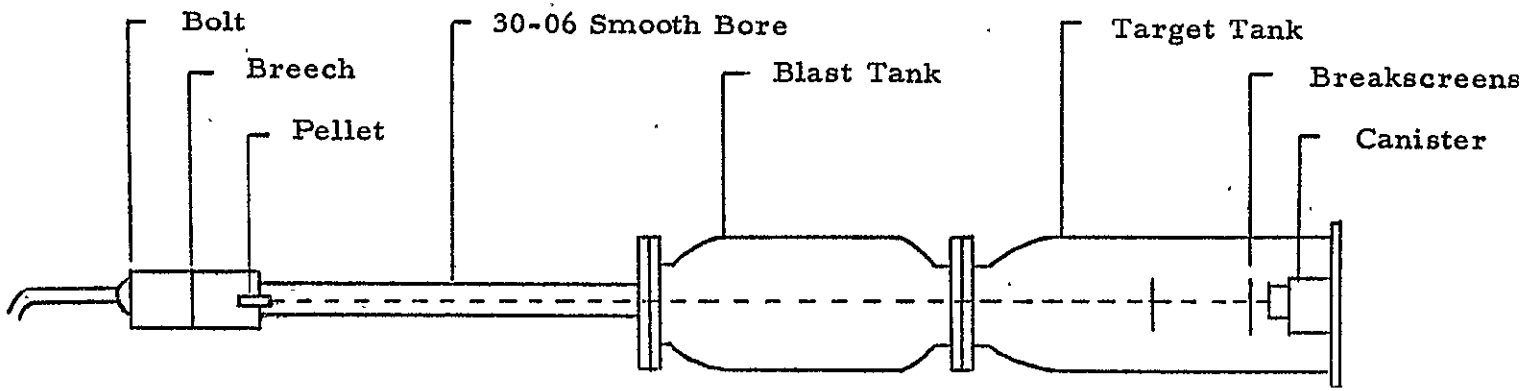

Figure 2: GAS AND POWDER GUN

canister. All particles of the impacted pellet were weighed again. The procedures used to recover the epoxy particles were identical to those employed in the analysis for released spores from seeded pellets after impact. The results of these firings established the percent of pellet recovered. The particles of each impacted pellet were saved for delivery to JPL.

3.3.2 Analysis for Released Spores After Pellet Impact

Analysis for the recovery of released spores was performed as outlined in Figure 3. The collection canister was opened in a Class 100 clean bench. Large Eccobond pellet particles were transferred, with sterile forceps, to a sterile petri plate. Trypticase Soy Agar (TSA) was then poured into the plate so that each particle was completely covered with the medium. Small particles were recovered from the canister by utilizing suction through a Millipore membrane field monitor unit. After thoroughly vacuuming the inside of each canister part, the Millipore unit was separated and the particles on the filter plated. In addition, TSA was poured into the Millipore unit and allowed to solidify.

The collection canister was reassembled and filled with 800 ml of sterile 0.1% peptone water. The cotton plug was replaced in the canister and the unit placed in an ultrasound bath so that the level of bath water was slightly above that of the peptone water. The canister was insonated for 30 minutes. The canister was removed from the bath, the pour spout opened, and the peptone wash water filtered through a Millipore membrane. The canister was rinsed with additional 0.1% peptone and the rinses also filtered. The canister was then opened and carefully examined for any remaining pieces of pellet. All membranes, particles, and field monitoring units were incubated in TSA for 2 weeks at 30°C. Daily examination was performed and each colony observed was recorded as one released spore.

3.3.3 Analysis for Total Number of Spores Surviving Impact

Analysis for the recovery of the total number of spores surviving impact was performed as outlined in Figure 3. The collection canister was opened and Eccobond particles removed with sterile forceps and by suction through

PELLET FABRICATION

PELLET FIRING

550, 1500, 3100 and 5100 feet/second

STAINLESS STEEL IMPACT

Analysis for Total Spore Level

1. Wet grind pellet in modified Waring blender.
2. Plate aliquots of ground Eccobond in TSA.
3. Incubation for 2 weeks at 30°C.
4. Plate count at 72 hours and 2 weeks.

Analysis for Spores Released After Impact

1. Canister opened.
2. Particles removed from canister by sterile forceps and suction through a Millipore membrane field unit.
3. Canister resealed.
4. 0.1% sterile peptone water added.
5. Unit placed in ultrasonic bath.
6. Millipore membrane filtering of peptone wash water.
7. Canister rinsed with 0.1% peptone and rinse water Millipore filtered.
8. Particles and membranes plated in TSA.
9. Incubation for 2 weeks at 30°C.
10. Daily examination.

Analysis for Total Spores Surviving Impact

1. Canister opened.
2. Particles removed from canister by sterile forceps and suction through a Millipore membrane field unit.
3. Particles embedded in 10% agar and molded into rod.
4. Rod wet ground in modified Waring blender.
5. TSA pour plates of ground epoxide incubated for 2 weeks at 30°C.
6. Plate count at 72 hours and 2 weeks.

Figure 3: TEST ANALYSIS

a Millipore membrane field unit. The particles were embedded in sterile 10% agar and molded into a rod. The agar rod containing the pellet particles was wet ground in a modified Waring blender that was fitted with a carborundum disc (Figure 4). After wet grinding, aliquots were plated in molten TSA and incubated for 2 weeks at 30°C. Daily examination was conducted and each colony thus observed was recorded as one spore surviving hard impact.

3.3.4 Analysis for Total Spore Level

On each day of firing two pellets were chosen at random to determine the total spore level. A pellet was placed in the modified Waring blender and wet ground. The contents of the blender were plated in molten TSA and incubated for 2 weeks at 30°C. This procedure established the number of spores present per gram of pellet.

3.3.5 Procedural Controls

On each test day, an unseeded control pellet was launched after the seeded pellets were fired. The control pellet particles were subjected to analysis as outlined in Figure 3. This control pellet established the reliability of the test data with respect to possible contamination due to procedural techniques.

Also, on each test day, a seeded pellet was selected at random and embedded in melted TSA. The plate was incubated at 30°C and examined periodically for 2 weeks. The absence of surface colonies established reliability in the method used to sterilize the pellet surface.

3.4 DATA RECORDING AND ANALYSIS

The data was recorded on data sheets that contained the date of pellet firing, pellet number and weight, the velocity of impact, the analysis performed and the results of the analysis. The data sheet for each test day was checked and initialed by the program manager.

These data provided information on: (1) the number of viable organisms released from solids after hard impact; (2) the percentage of viable microorganisms surviving impacts at the test velocities; (3) differences in release and/or, survival of organisms due to variations in impact velocities.

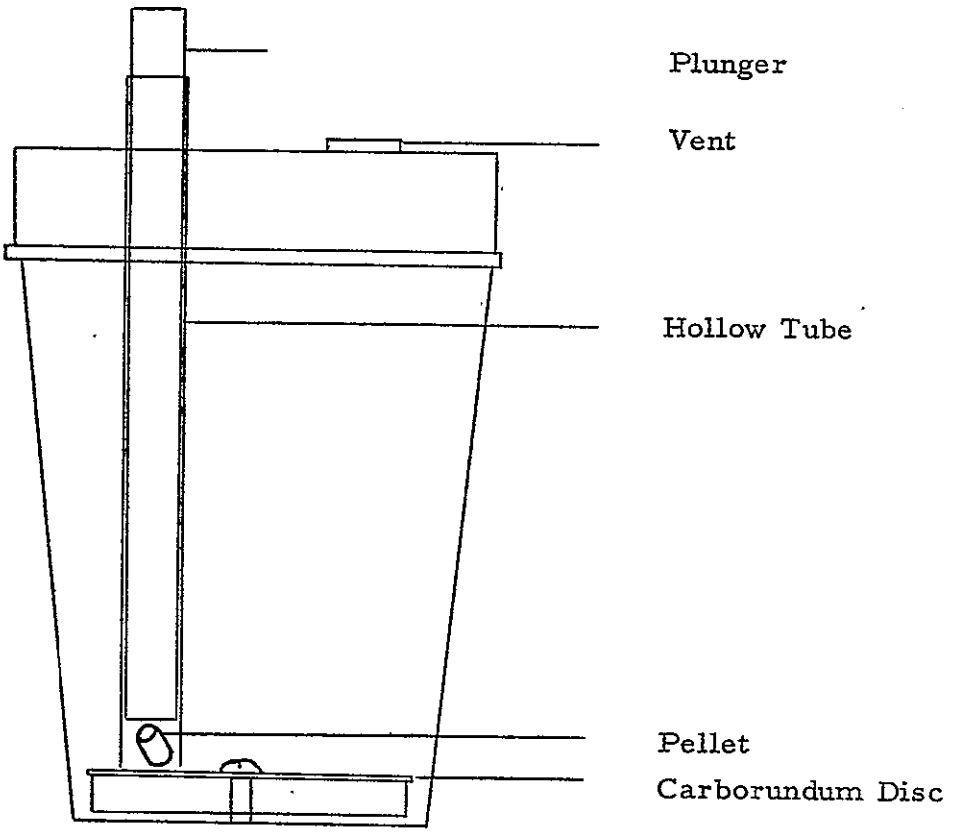

Figure 4: MODIFIED WARING BLENDER

The data was evaluated statistically using analysis of variance techniques. These analyses detected any significant variance between pellets in a replicate group and between pellets impacted at 550, 1500, 3100 and 5100 ft/sec.

4.0 RESULTS

4.1 PERCENT PELLET RECOVERY AFTER IMPACT

The percentage of epoxy recovered from the canister after pellet impaction at the four test velocities is presented in Table 2.

Table 2: PERCENT RECOVERY OF PELLET FOLLOWING IMPACT

Velocity Ft/Sec.	Mean Percent Recovery of Pellet (a)
550	97.
1500	95.
3100	91.
5100	61.

(a) Mean of 6 pellets

At 550, 1500, and 3100 ft/sec., virtually all of the pellet was recovered. At the higher velocity, 5100 ft/sec, only 61% of the initial weight of a pellet could be reclaimed.

An impact velocity of 550 and 1500 ft/sec produced completely shattered pellets. The fractures had clean, sharp edges. At 3100 and 5100 ft/sec, a large amount of the epoxy pellet appeared to powder at impact. Figures 5, 6, 7, and 8 show a comparison of the pellet fracturing patterns at the four test velocities.

4.2 SPORES RELEASED FROM THE INTERIOR OF IMPACTED PELLETS

The percentage of spores released from the interior of the epoxy pellets on hard impact is presented in Table 3.

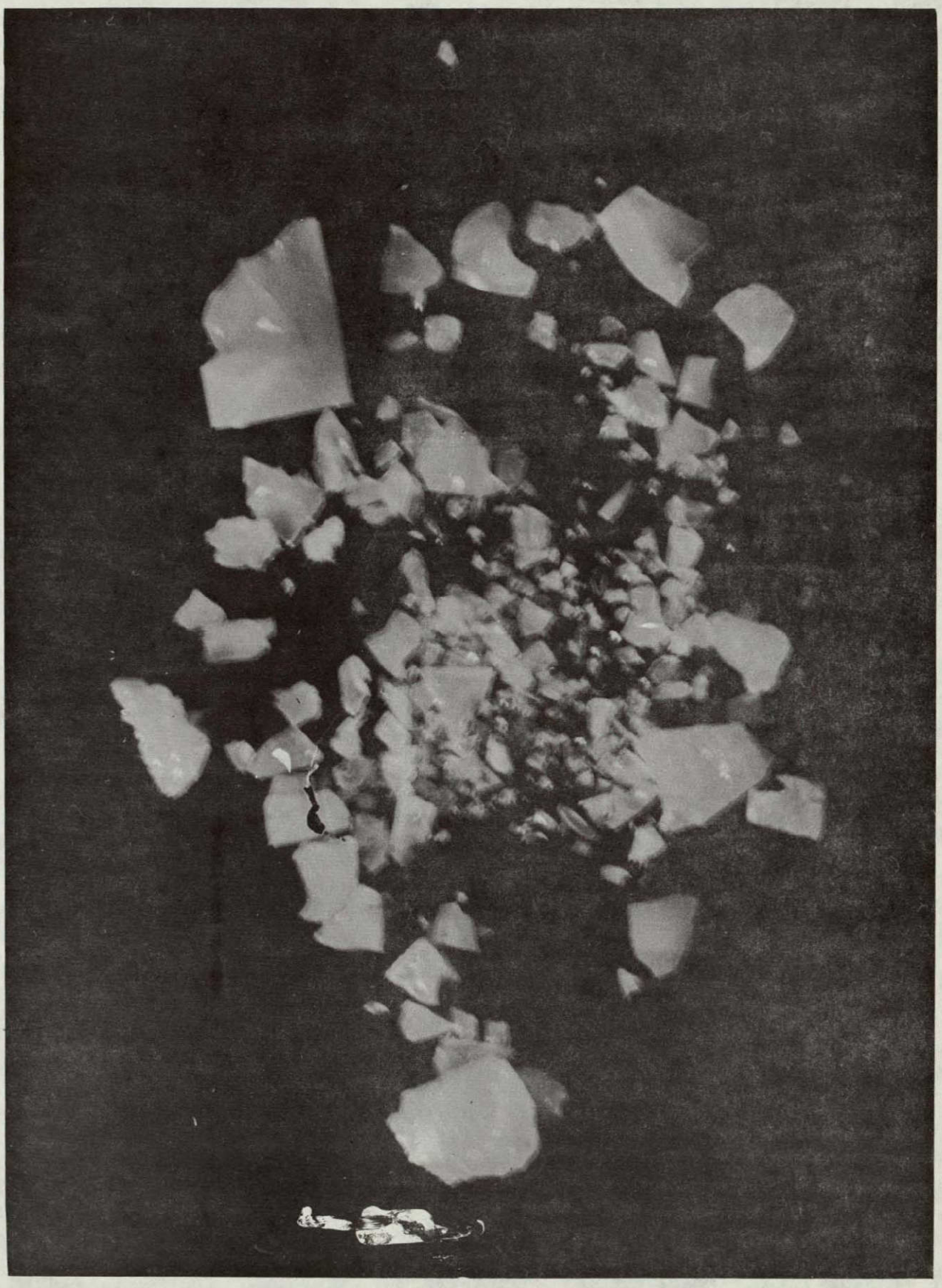

Figure 5: ECCOBOND EPOXY PELLET AFTER IMPACTION ON STAINLESS STEEL AT 550 FT/SEC

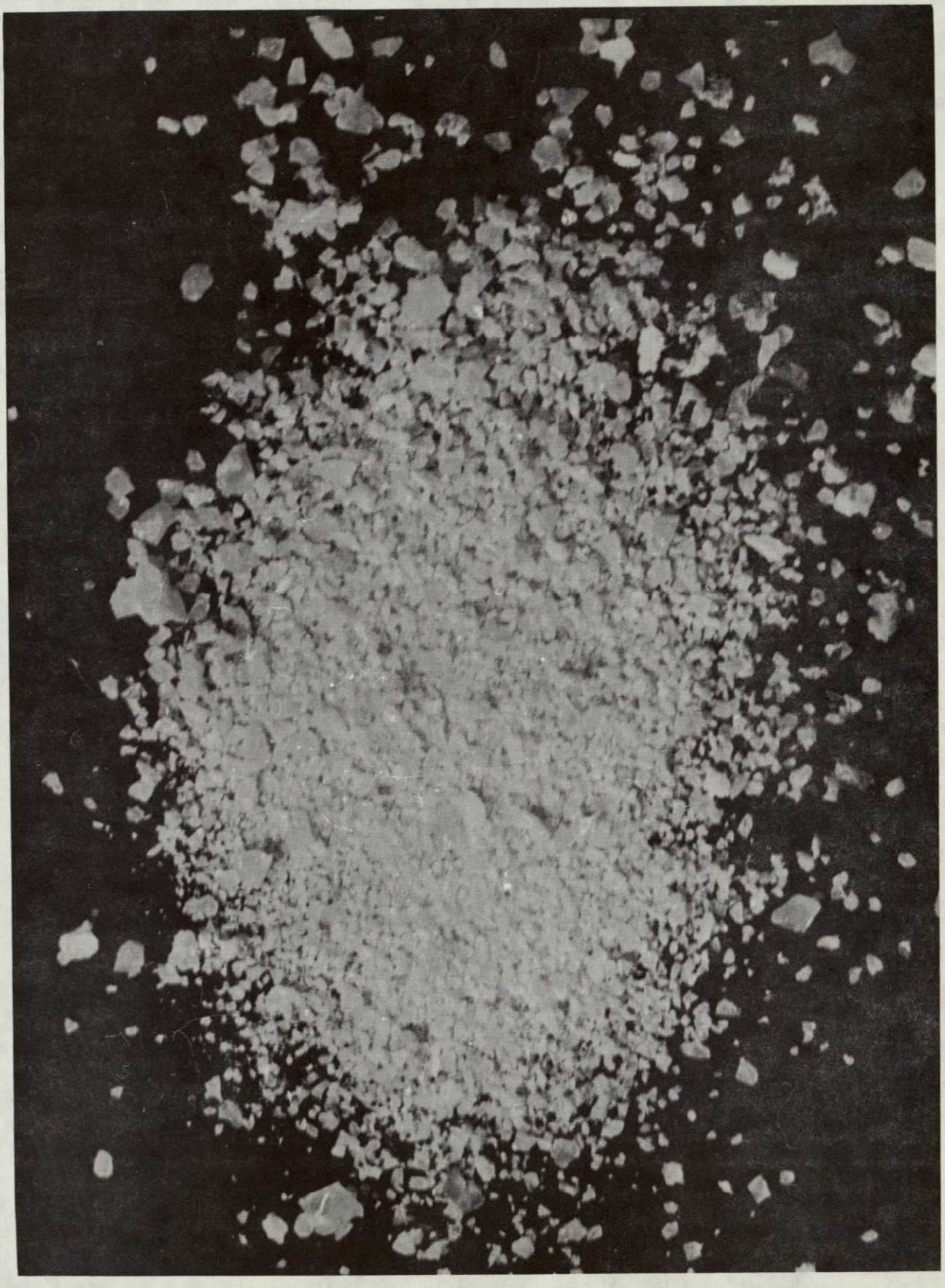

Figure 6: ECCOBOND EPOXY PELLET AFTER IMPACTION ON STAINLESS STEEL AT 1500 FT/SEC

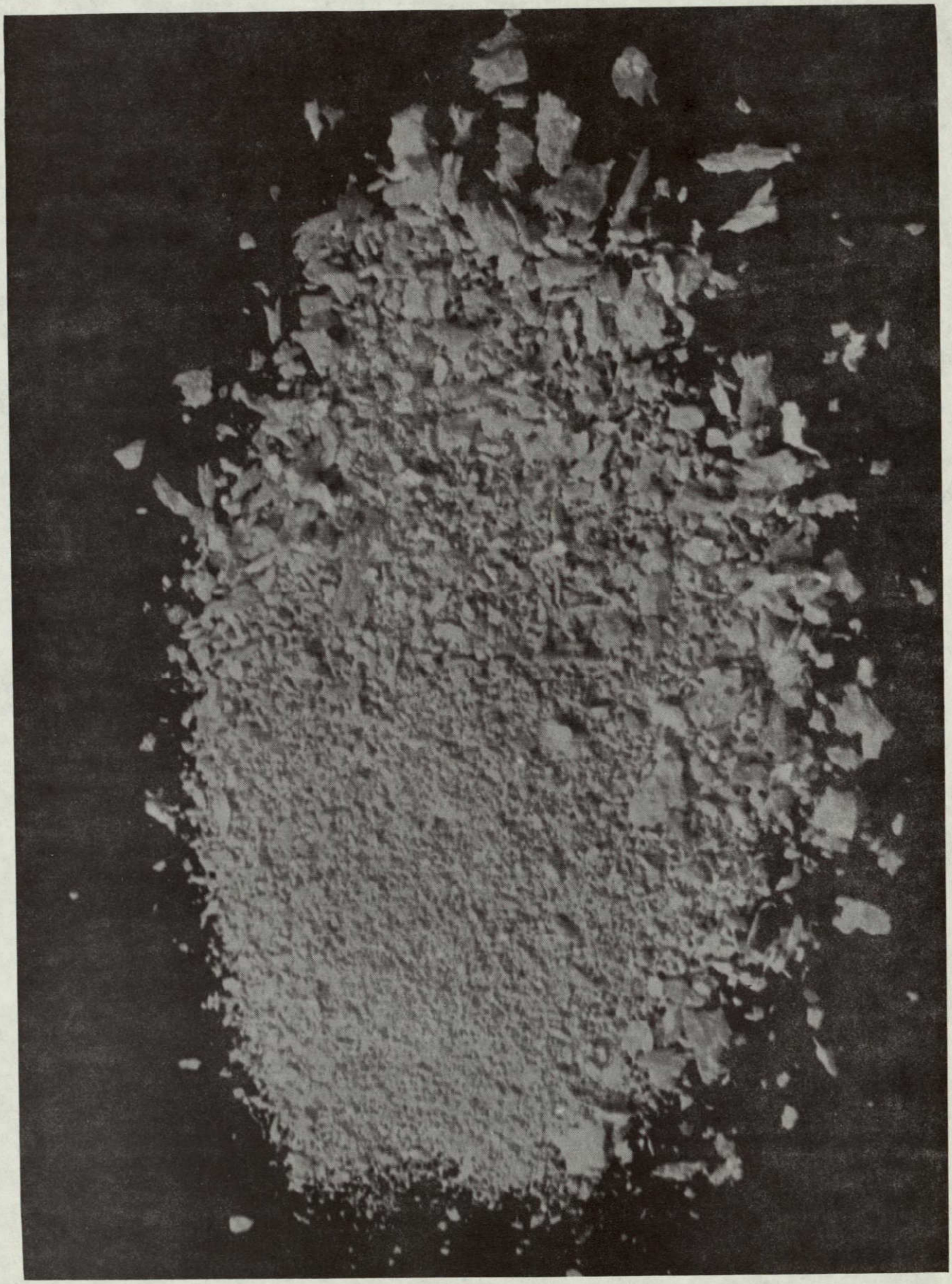

Figure 7: ECCOBOND EPOXY PELLET AFTER IMPACTION ON STAINLESS STEEL AT 3100 FT/SEC

Figure 8: ECCOBOND EPOXY PELLET AFTER IMPACTION ON STAINLESS STEEL AT 5100 FT/SEC

Table 3: SPORES RELEASED FROM THE INTERIOR OF INTERNALLY CONTAMINATED PELLETS AFTER IMPACTION

Velocity Ft/Sec.	Mean Percent Spores Released (a) (10^3 Spores Initially Available for Release)
550	6.2
1500	22.9
3100	12.7
5100	1.1

(a) Mean of 6 pellets

4.3 EFFECT OF IMPACT ON SPORE SURVIVAL

The percentage of viable spores recovered after hard impact of Eccobond pellets at the four test velocities is shown in Table 4.

Table 4: EFFECT OF IMPACT UPON THE SUBSEQUENT SURVIVAL OF SPORES IN THE INTERIOR OF AN EPOXY MATERIAL

Velocity Ft/Sec	Mean Percent Spores Surviving Impact (a)
550	81
1500	80
3100	91
5100	6

(a) Mean of 6 pellets

4.4 TEST CONTROLS

No background contamination due to fabrication and handling of the epoxide material, or due to the recovery and analytical procedures was observed. In addition, the surface sterilization method was found to eliminate all external spores from the projectiles.

4.5 STATISTICAL ANALYSIS

An applicable analysis of variance technique was employed to assess the data obtained on spore release. The results show a highly significant difference ($F = 37.7$ with 3 and 20 degrees of freedom) in the spore percentage release at the four velocities. The differences were examined at the 1% level. A second analysis of variance was conducted to investigate spore survival after impact. This analysis revealed a highly significant effect ($F = 19.3$ with 3 and 20 degrees of freedom) of velocity on the percentage of spore survival after impact. These differences were significant at the 1% level.

5.0 DISCUSSION AND SUMMARY

Eccobond epoxy pellets were fabricated so that each pellet interior contained 10^3 B. subtilis var. niger spores. A series of these pellets were launched from a gun at 550, 1500, 3100 and 5100 ft/sec. Impact occurred on a stainless steel plate mounted in a collection canister. After impact, the pellet fragments were analyzed to determine the number of released viable microorganisms and also to determine the effect of impact upon survival of spores.

The analysis of Eccobond survival data showed a highly significant effect of velocity on the percentage spore survival. These results were comparable to those obtained in previous tests. These comparable results occurred even though spore survival remained relatively high up through velocities of 3100 ft/sec (Table 4). The plateau in survival through 3100 ft/sec may partially explain the relatively high release rates seen at 1500 and 3100 ft/sec in Table 3. Plateaus of this magnitude have not been obtained before. Previous survival data have shown linear relationships with respect to velocity.

An analysis of the Eccobond release data shows only a one log reduction in the number of microorganisms released when compared to the initial number of organisms present before firing. Also, the percentage differences between firing velocities, with respect to release, were found to be highly significant by statistical tests. Neither of these results are comparable to data obtained from previous investigations. Therefore, it appears that either the microbial release characteristics of Eccobond epoxy are significantly different from those of methyl methacrylate or problems exist in the assay procedure.

In order to accurately calculate the percentage of microbial release from Eccobond at hard impact, the total number of spores available for release must be established. Since epoxy resins are impervious to solvents, a potential problem exists when enumeration of pellet spore levels is performed by pellet grinding. The number of spores added to the Eccobond during fabrication totaled 10^5/gram of epoxy. After pellet fabrication, the grinding procedure showed the spore level/gram pellet to be approximately 10^3.

The approximate two log reduction in number of organisms inoculated and number of spores recovered may have been due to either effects of Eccobond polymerization, the inability of the grinding process to render particle sizes small enough to liberate all spores, or a combination of these two factors. A 10^5 inoculum level when used to seed methyl methacrylate, will show only a one log reduction when the spores are recovered by plastic dissolution.

The possibility that pellets were consistently launched with a higher spore level than the 10^3/gram established by grinding must be considered when evaluating data such as shown in Table 3. The relatively high percentage of microbial release may be explained if, in actuality, more spores were available for release than the assay procedure showed. Even a one log increase in numbers initially present would decrease the percent released by a factor of ten. The results then would be more consistent with previous data on the percentage of microbial release from solids (Section II and III).

Before final conclusions can be reached on the Eccobond release data, an investigation must be conducted to establish an efficiency factor for the grinding technique as a method of releasing microorganisms from solids for enumeration. The grinding efficiency factor should also be correlated to the dissolution technique used for methyl methacrylate. A discussion of the currently available epoxy data in relationship to planetary quarantine considerations is included in Section I of this report.

6.0 APPENDIX

6.1 MATERIALS AND EQUIPMENT

6.1.1 Eccobond 55 - Emerson & Cuming

Eccobond 55 and Catalyst #9 were used to fabricate the epoxide pellets.

6.1.2 Chlorine

A dilution of Chlorox bleach in distilled water was prepared to effect a final solution of 2000 ppm chlorine and this solution was mixed fresh when needed. The solution was used to surface sterilize the projectiles.

6.1.3 Thiosulfate Solution

A 2% solution of sodium thiosulfate in distilled water was prepared, Millipore membrane filter sterilized, and stored in a sterile screw capped bottle. It was used the same day it was prepared. The solution was employed to neutralize any residual chlorine on the pellet surface.

6.1.4 Millipore Membranes

Millipore membranes (Millipore Corp., Bedford, Mass.) 0.47 mm diamter, 0.45 μ pore size were autoclaved for 20 minutes at 121°C. Two filters were selected at random from each package and plated in TSA to monitor for sterility. The Millipore filters were used to recover spores from peptone water canister washings.

6.1.5 Trypticase Soy Agar

Twenty grams of Trypticase Soy Agar (Baltimore Biological Laboratories) were placed in a one liter screw capped bottle containing 500 mls distilled water. Complete suspension of the agar was attained by shaking the bottle. The bottle was labelled with autoclave tape and sterilized. Twenty-four bottles of TSA were autoclaved for 30 minutes at 121°C. After the sterilization cycle, the TSA was stored in a 50°C dry heat oven at 50°C. TSA was used as the nutrient medium for the enumeration of viable spores.

6.1.6 Peptone Water

Peptone (Difco) was added to distilled water to effect a final concentration of 0.1%. Twenty-four one liter screw capped bottles were filled with 800 mls of peptone water, labelled with autoclave tape and autoclaved for 30 minutes at 121°C. After sterilization, the bottles were allowed to cool to room temperature and stored in a cabinet until used.

6.1.7 Delta Ultrasonic Bath

An ultrasonic generator, Model DS-825, and an ultrasonic tank, Model NT-17(12) (Delta Sonics) was employed in the test program. The equipment has a power output of 850 watts average and 1700 watts peak. The output frequency is rated at 25 KC $\pm$ 3 KC. The low power setting delivers 60% maximum power and high power delivers maximum power.

6.1.8 Autoclave

A Wilmot Castle Thermatic 60, automatic autoclave, was available for the sterilization of materials used in the test program.

6.1.9 Miscellaneous Laboratory Equipment

All glassware, rubber stoppers, Millipore holders, forceps, magnetic stirring bars, collection canisters, and other laboratory equipment that was used in the study was autoclaved at 121°C for a sufficient period to render the objects sterile. Materials that were unable to withstand excessive heat were either sterilized with ethylene oxide gas or by surface chemical treatment. In addition, glassware was dried after sterilizing by holding the object in a dry heat oven until observable moisture disappeared.

6.2 UNREDUCED PROGRAM DATA

The unreduced data from Test III is presented in Tables 5 through 8.

Table 5 : PERCENT PELLET RECOVERY OF ECCOBOND PELLETS FOLLOWING IMPACT ON STAINLESS STEEL

Pellet Number	Velocity Ft/Sec	Weight Before Firing, Grams	Weight After Firing, Grams	Percent Recovered
3B-1-1	553	0.959	0.951	99.2
3B-1-2	547	0.959	0.940	98.0
3B-1-3	545	0.957	0.893	93.3
3B-1-7	553	0.957	0.957	100.0
3B-1-8	550	0.958	0.923	96.3
3B-1-9	597	0.958	0.935	97.6
3B-1-13	1580	0.963	0.912	94.7
3B-1-14	1660	0.962	0.932	96.9
3B-1-15	1475	0.957	0.928	97.0
3B-2-7	1585	0.959	0.902	94.1
3B-2-8	1565	0.958	0.905	94.5
3B-2-9	1560	0.960	0.915	95.3
1	3300	0.960	0.860	89.6
2	3240	0.960	0.836	87.1
3	3230	0.960	0.882	91.9
4	3110	0.960	0.911	94.9
5	2930	0.960	0.886	92.3
6	3070	0.960	0.856	89.2
7	5100	0.960	0.830	86.5
8	5100	0.960	0.694	72.3
9	5100	0.960	0.499	52.0
10	5100	0.962	0.651	67.7
11	5100	0.961	0.494	51.4
12	5100	0.960	0.353	36.8

Table 6 : TOTAL SPORE LEVEL OF ECCOBOND CONTROL PELLETS

Pellet Number	Pellet Weight, Grams	Spore Count	Spore Count/ Gram Pellet	Total Spore Level/Gram Pellet (a)	Plastic Batch
3B-1-22	0.936	2,407	2,572		
3B-1-21	0.960	3,422	3,565		
3B-1-20	0.960	3,429	3,572	3,137	3B-1
3B-1-19	0.961	3,603	3,749		
3B-1-18	0.961	2,277	2,369		
3B-1-17	0.960	2,878	2,998		
3B-2-22	0.956	2,813	2,943		
3B-2-21	0.959	3,531	3,682	2,394	3B-2
3B-2-20	0.959	761	794		
3B-2-19	0.958	2,066	2,157		
3B-3-22	0.942	1,721	1,827		
3B-3-21	0.959	1,653	1,724	1,537	3B-3
3B-3-18	0.961	1,508	1,569		
3B-3-17	0.958	986	1,029		
3B-5-15	0.971	1,063	1,095	1,243	3B-5
3B-5-14	0.972	1,353	1,392		

(a) Mean for that plastic batch

Table 7 : SPORES RELEASED FROM INTERNALLY CONTAMINATED ECCOBOND AFTER IMPACT ON STAINLESS STEEL

Velocity Ft/Sec.	Pellet Number	Pellet Weight, Grams	Initial Spore Level/G Pellet Before Impact	Number Spores Released Upon Impact	Number Spores/G Pellet Released Upon Impact	% Spores Released
553	3B-1-1	0.959	3137	125	130	4.2
547	3B-1-2	0.959	3137	120	125	4.0
545	3B-1-3	0.957	3137	138	144	4.6
553	3B-1-7	0.957	3137	242	253	8.1
5550	3B-1-8	0.958	3137	248	259	8.3
597	3B-1-9	0.958	3137	245	256	8.2
1580	3B-1-13	0.963	3137	487	506	16.1
1660	3B-1-14	0.962	3137	623	648	20.6
1475	3B-1-15	0.957	3137	492	514	16.4
1585	3B-1-7	0.959	3137	749	781	32.6
1565	3B-1-8	0.958	3137	661	690	28.8
1560	3B-1-9	0.960	3137	519	541	22.6
3180	3B-2-16	0.959	2394	189	197	8.2
2970	3B-2-17	0.959	2394	354	369	15.4
3120	3B-3-1	0.961	1537	184	191	12.5
3040	3B-3-2	0.961	1537	188	196	12.7
3200	3B-3-3	0.960	1537	206	215	14.0
3150	3B-3-4	0.959	1537	198	206	13.4
5100	3B-3-5	0.960	1537	5	5	0.3
5100	3B-3-6	0.960	1537	7	7	0.5
5100	3B-3-7	0.961	1537	15	16	1.0
5100	3B-3-8	0.962	1537	11	11	0.7
5100	3B-3-9	0.965	1537	42	44	2.8
5100	3B-3-10	0.963	1537	18	19	1.2

Table 8 : EFFECT OF IMPACT ON STAINLESS STEEL UPON THE SUBSEQUENT SURVIVAL OF SPORES IN THE INTERIOR OF ECCOBOND PELLETS

Velocity Ft/Sec	Pellet Number	Pellet Weight, Grams	Initial Spore Level/G Pellet Before Impact	Number Spores Surviving Impact	Number Spores/G Pellet Surviving Impact	% Spores Surviving Impact
544	3B-1-4	0.958	3137	2726	2846	90.7
592	3B-1-5	0.959	3137	3002	3130	99.9
550	3B-1-6	0.957	3137	1240	1296	41.3
575	3B-1-10	0.957	3137	3147	3288	104.8
537	3B-1-11	0.959	3137	3582	3735	119.1
545	3B-1-12	0.958	3137	964	1006	32.1
1665	3B-1-16	0.959	3137	2978	3105	99.0
1595	3B-2-1	0.959	2394	1254	1308	54.6
1625	3B-2-2	0.960	2394	2088	2175	90.9
1600	3B-2-3	0.959	2394	1711	1784	74.5
1625	3B-2-4	0.959	2394	2088	2177	91.0
1680	3B-2-5	0.961	2394	1599	1664	69.5
2900	3B-5-1	0.970	1243	914	942	75.8
2960	3B-5-2	0.972	1243	1407	1448	116.4
2920	3B-5-3	0.972	1243	1363	1402	112.8
3010	3B-5-4	0.972	1243	1044	1074	86.4
2970	3B-5-5	0.972	1243	856	881	70.8
2960	3B-5-6	0.971	1243	1030	1061	85.3
5100	3B-3-11	0.962	1537	96	100	6.5
5100	3B-3-12	0.962	1537	201	209	13.6
5100	3B-3-13	0.961	1537	69	72	4.7
5100	3B-3-14	0.964	1537	33	34	2.2
5100	3B-3-15	0.962	1537	36	37	2.4
5100	3B-3-16	0.962	1537	66	69	4.5